ARKIV FÖR KEMI Band 18 nr 4

Mitgeteilt am 8. Februar 1961 von Arne Fredga und Gunnar Blix

Infrarotspektren sämtlicher stellungsisomerer Monohydroxy- und Monoketo-oktadekansäuren und deren Methylester

I. Monoketo-oktadekansäuremethylester[1]

Von Ingrid Fischmeister[2]

Mit 9 Abbildungen im Text

Die Spektren kristalliner langkettiger Fettsäuren (mit gerader Methylenkette) sind im Gebiet von ca. 1350–1150 cm^{-1} durch regelmäßig angeordnete Banden, deren Anzahl mit der Kettenlänge variiert, gekennzeichnet. Für die längeren Glieder beträgt sie nach Meiklejohn, Meyer, Aronovic, Schuette und Meloch [1] etwa die Hälfte der C-Atome. Diese Banden wurden von Sinclair, McKay, Jones und Myers [2, 3, 4] zuerst beobachtet und in Analogie zu ähnlichen in Kohlenwasserstoffspektren [5] den Methylen-wagging und/oder twisting-Schwingungen zugeordnet. Etwa gleichzeitig berechneten Primas und Günthard [6, 7] die Frequenzen dieser sowie der Methylen bending-, rocking- und der C–C-Valenzschwingungen für die von ihnen untersuchten langkettigen Dikarbonsäureester [8] und fanden für alle Schwingungen Reihen von Banden, die mit der Anzahl der Methylengruppen wachsen (in Übereinstimmung mit den an den Kohlenwasserstoffen gefundenen Bandenserien).

Substituenten in der Methylenkette zerstören diese regelmäßige Anordnung von Banden [3] und in den Monoketo-oktadekansäuremethylestern sind die vorhandenen Banden hauptsächlich durch den Kettenteil zwischen Ester- und Ketogruppe hervorgerufen [9]. Kürzlich beschrieb Susi [10], daß auch Doppelbindungen die Kette unterbrechen und nur mehr der eine Teil zu den Banden zwischen 1350 und 1180 cm^{-1} beiträgt, während Hydroxylgruppen die Kette nicht unterbrechen, dafür aber Unregelmäßigkeiten hervorrufen.

Von Bergström, Aulin-Erdtman, Rolander, Stenhagen und Östling [11] wurden im Zusammenhang mit Arbeiten über die Autoxydation ungesättigter Fettsäuren sämtliche stellungsisomeren Monohydroxy- und Monoketo-oktadekansäuren synthetisiert. Dies ergab die Möglichkeit, den Einfluß dieser zwei Substituenten in ihren verschiedenen Stellungen auf die Spektren zu studieren. Weiters war interessant zu untersuchen, ob die Spektren zur Identifikation der einzelnen Substanzen dienen können, da eine solche nur mit Hilfe des thermischen Verhaltens und der Röntgen-

[1] Vorgetragen auf der Tagung der Schwedischen Biochemischen Gesellschaft in Uppsala am 28.1.1956.

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daten schwierig ist. Seit kurzem wird allerdings auch die Massenspektrometrie mit Erfolg zur Identifikation herangezogen [12].

Unter den Monoketo-oktadekansäuremethylestern wurde Polymorphie nur beim 3-Ketoester beobachtet. Von den Monoketo-oktadekansäuren sind einige polymorph, während bei den Monohydroxy-oktadekansäuren und -estern Polymorphie häufig ist [11]. Erstere werden wegen ihres einfachen Verhaltens zuerst besprochen.

Experimentelles

Synthese und Reinheit der Substanzen sind in Ref. [11] beschrieben. Die Spektren wurden auf einem Perkin–Elmer Spektrophotometer Modell 21 mit Natriumchloridprisma aufgenommen. Die kristallinen Substanzen sind in Kaliumbromid gepreßt untersucht. Zur Herstellung einer Kaliumbromidplatte werden 3 mg Substanz mit 300 mg gefriergetrocknetem Kaliumbromid von Hand ca. 3 Minuten lang in einem Achatmörser vermischt und dann im Vakuum mit einem Druck von 13 t/cm² etwa 3 Minuten lang gepreßt. Umwandlung in eine andere polymorphe Form wird durch Erwärmen der Kaliumbromidplatte über den Schmelzpunkt der Substanz und Abkühlenlassen durchgeführt. Die dabei auftretende Trübung wird durch Pulverisieren und erneutes Pressen entfernt. Eine eingehendere Beschreibung des Verfahrens findet sich in [13].

Diskussion der Spektren

Die Spektren der 16 Stellungsisomeren sind in Abb. 6 bis 9 am Ende der Arbeit wiedergegeben. Aus ihnen und aus den schematischen Darstellungen in Abb. 1 und 4 ist ersichtlich, daß die Banden der C = O-Valenzschwingung (ν(C = O)), der Ester-C–O-Schwingung (ν(C–O)) und der Methyl- und Methylen-bending-Schwingungen ($\delta(CH_3)$ und $\delta(CH_2)$) in den meisten Spektren (2- und 3-Ketoester bilden eine Ausnahme) gleiche Lagen haben. Die Banden der Methylen-wagging- und -twisting-Schwingungen ($\gamma_w(CH_2)$ und ($\gamma_t(CH_2)$) sowie der rocking-($\gamma_r(CH_2)$)-Schwingungen unterscheiden sich dagegen von Spektrum zu Spektrum; ihre Frequenzen, als Funktion der Lage des Substituenten aufgetragen, liegen auf Scharen verwandter Kurven.

Bereich der C = O-Valenzschwingungen

Die Lagen der Ester- und Ketokarbonylbanden sind, mit den in Klammern angegebenen Abweichungen, folgende: $\nu(C=O)_{Estergruppe}$: 1742–1740 cm^{-1} (17- und 3-Ketoester 1733 bzw. 1751 cm^{-1}) und $\nu(C=O)_{Ketogruppe}$: 1712–1709 cm^{-1} (3-, 6-, 15- und 16-ketoester haben das Maximum bis zu 1721 cm^{-1} verschoben). Der 3-Ketoester hat normale Lage der Karbonylbanden und ist daher im festen Zustand nicht enolisiert. In Lösung ist er offenbar teilweise enolisiert, da neben den Banden bei 1718 und 1742 cm^{-1} schwache bei 1629 und 1650 cm^{-1} auftreten, die charakteristisch für konjugierte Chelate sind.

Bereich der CH_2- und CH_3-Beugungsschwingungen (1480–1380 cm^{-1})

Zwischen 1480 und 1380 cm^{-1} treten vier Banden auf, die folgende Zuordnung haben: bei 1477–1464 cm^{-1} die Bande der $\delta(CH_2)$-Schwingung, der die asymmetrische $\delta(CH_3)$-Schwingung überlagert ist; bei 1440 cm^{-1} vermutlich eine Bande der $-\overset{\overset{O}{\|}}{C}-O-$-Gruppe (ähnlich der in Säuren auftretenden [14]), bei 1420–1410 cm^{-1} die

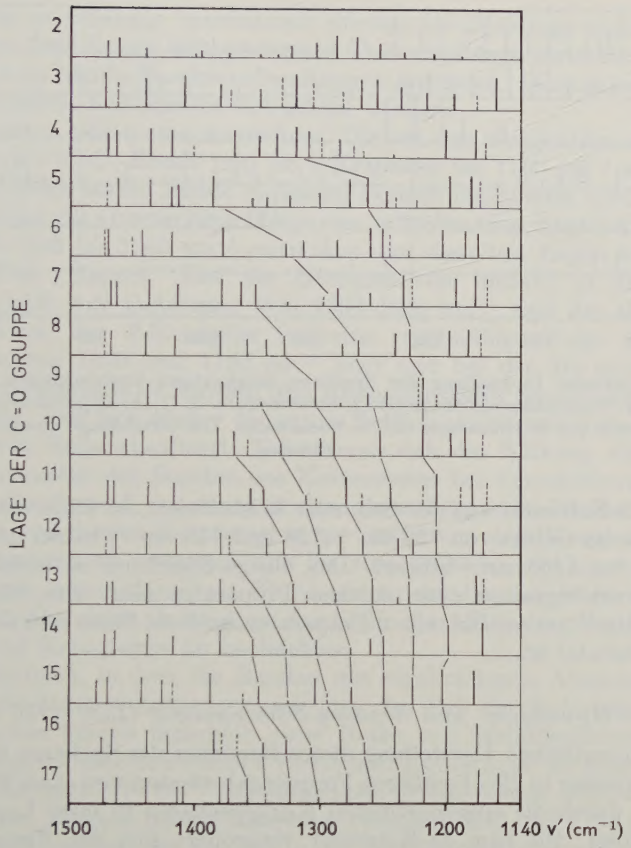

Abb. 1. Schematische Darstellung der Monoketo-oktadekansäuremethylesterspektren von 1500–1140 cm^{-1}. Die Strichlänge entspricht der Stärke der Banden.

Bande der $\delta(CH_2)$-Schwingung der zur Estergruppe α-ständigen Methylengruppe [15, 2], und bei 1390–1380 cm^{-1} die Bande der symmetrischen $\delta(CH_3)$-Schwingung.

Die $\delta(CH_2)$-Bande bei 1477–1464 cm^{-1} ist, außer im 3-Ketoester, breit und entweder in zwei Maxima aufgespalten, oder sie hat neben dem Hauptmaximum eine starke Schulter. In beiden Fällen tritt sie zusammen mit einer doppelten $\gamma_r(CH_2)$-Bande auf (729 und 719 cm^{-1}). Diese gemeinsame Verdopplung wurde zuerst von Stein und Sutherland [16] an Polyäthylen beobachtet. Beim 3-Ketoester sind sowohl die $\delta(CH_2)$- als auch die $\gamma_r(CH_2)$-Bande einfach und liegen bei 1475 und 719 cm^{-1}; sie sind damit ein weiteres Beispiel dafür, daß die längerwellige rocking-Bande immer zusammen mit der kürzerwelligen bending-Bande auftritt [13]. Dieses letztgenannte Paar wird, wie der Vergleich mit den Kristallstrukturen bei den Methylfettsäuren (17) ergab, bei paralleler Anordnung der Zick-zack Ebenen der Methylenketten in der Elementarzelle (gewöhnlich in trikliner Packung) beobachtet, während beide Paare gleichzeitig auftreten, wenn die Ebenen der Methylenketten senkrecht zueinander angeordnet sind (gewöhnlicher orthorhomischer Packungstyp).

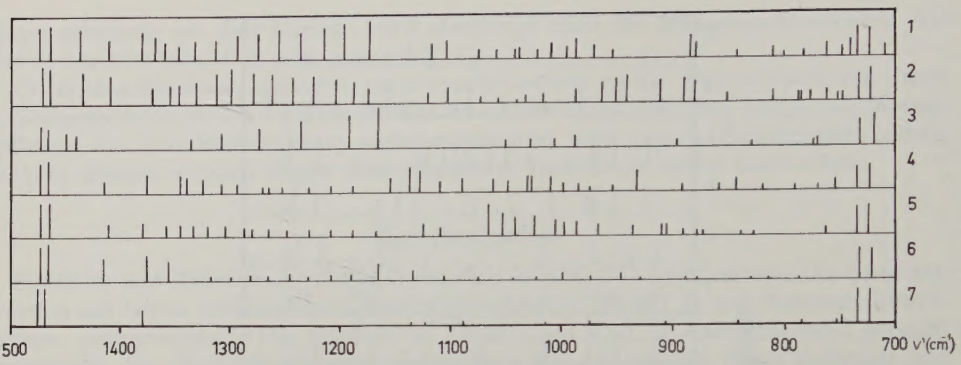

Abb. 2. Schematische Darstellung der Spektren langkettiger Verbindungen mit verschiedenen Endgruppen. 1: Stearinsäuremethylester. 2: Stearinsäure. 3: (1,18)-Dijodoktadekan. 4: Eikosanol-(2). 5: Heneikosen-(2). 6: Eikosanon-(2). 7: Nonadekan. Die Strichlänge entspricht der Stärke der Banden.

Wird der 3-Ketoester aus der Schmelze kristallisiert, so kann sein Spektrum eine zweite Bande im Gebiet von 720 cm^{-1} (728 und 718 cm^{-1}) haben und gleichzeitig ist die Schulter bei 1466 cm^{-1} stärker. Das übrige Spektrum ist nicht verändert. Das Röntgenpulverfotogramm eines solchen Präparates zeigt eine Beimischung einer anderen Kristallform an. Bei reinen Präparaten ist diese Form sehr schwer zu erhalten da sie sehr instabil ist.

Bereich der CH_2-wagging- und -twisting-Schwingungen (1380–1150 cm^{-1})

In der schematischen Darstellung dieses Bereiches der Spektren in Abb. 1 sind die beim 17-Ketoester in gleichmäßigen Frequenzabständen von etwa 20 cm^{-1} auftretenden Banden durch die eingezeichneten Kurvenscharen in ihrer Lage bis zum 4-Ketoester verfolgt. Bis zum 13-Ketoester vergrößert sich der Frequenzabstand auf etwa 30 cm^{-1}. Vom 12-Ketoester ab treten so häufig Bandenaufspaltungen auf, daß keine Angabe des Abstandes mehr möglich ist und beim Verfolgen der Kurven die stärksten Banden einer Gruppe genommen wurden.

Diese Banden sind bei ca 1380 cm^{-1} durch die Bande der symmetrischen $\delta(CH_3)$-Schwingung und bei 1180–1170 cm^{-1} von der Ester-(C–O)-Bande begrenzt. Solche Kurvenscharen sind für homologe Reihen mit zunehmender Länge der Methylenkette charakteristisch [3, 8, 18, 1, 10]; man findet sie in den Spektren homologer Fettsäuren bei etwa denselben Wellenzahlen. In unserem Fall ist aber die Länge des Gesamtmoleküles konstant. Länger wird (vom 4- bis zum 17-Ketoester) nur der Kettenteil zwischen Ester- und Ketogruppe, sodaß die hier gefundenen Regelmäßigkeiten in den Bandenfolgen durch diesen Teil der Kette hervorgerufen sein müssen. Der Methylenkette zwischen Keto- und Methylgruppe kommt offenbar untergeordnete Bedeutung zu, da für sie keine derartigen Serien gefunden werden.

Der Grund dafür liegt darin, daß die Intensitäten dieser Banden allgemein von den Kettenendgruppen abhängig sind. Bei der verwendeten Aufnahmekonzentration absorbieren Ester, Säuren und Halogenide stark, Ketone, Alkohole und Alkene schwach und Alkane nicht merkbar (siehe Abb. 2). Die beiden Teile der Methylenkette benehmen sich also so, als ob sie einem Ester bzw. einem Keton zugehörten. Die Ketogruppe teilt somit das Molekül hinsichtlich seines Absorptionsverhaltens in

zwei Teile. Völlig unabhängig voneinander dürften sie allerdings nicht sein, denn sonst würden bei den Estern mit geringerer Teilkettenlänge zwischen Ester- und Ketogruppe nicht so häufig Bandenaufspaltungen auftreten. Diese müssen wohl als Störungen des anderen Kettenteiles betrachtet werden.

3- und 2-Ketoester bilden eine Ausnahme. Die bei den übrigen Estern bei 1180–1170 cm^{-1} liegende ν(C–O)-Bande tritt im 3-Ketoester bei 1160 cm^{-1} auf, während beim 2-Ketoester keine starke Bande in diesem Bereich beobachtet wird. Die Bande bei 1160 cm^{-1} bildet die Grenze einer Folge von mittelstarken, regelmäßig angeordneten Banden die sich bis 1260 cm^{-1} erstrecken und dieselben Lagen haben wie im 17-Ketoester. (Der „längere" Teil der Methylenkette umfaßt in diesen beiden Isomeren auch gleich viel C-Atome.) Von 1380–1260 cm^{-1} sind die Banden dann unregelmäßig. Auch der 2-Ketoester hat eine regelmäßige Folge mittelstarker Banden, die zwischen 1330 und 1190 cm^{-1} liegt und bei der die mittlere Bande (bei 1290 cm^{-1}) die stärkste ist. Anzahl und Intensität dieser regelmäßig angeordneten Banden zeigen, daß hier wieder die ganze Methylenkette mit Keto- und Estergruppe als polarem Ende absorbiert. Denkt man sich die Wirkung dieses polaren Endes auf die Intensität der Banden des Kettenrestes bei Verschiebung der Ketogruppe langsam abnehmend, so wären in den darauffolgenden Gliedern schwächer werdende Banden von diesem Kettenrest sowie starke von dem Kettenteil zwischen Ester- und Ketogruppe zu erwarten. Die bis zum 13-Ketoester hin beobachteten Unregelmäßigkeiten wären damit als Überlagerung der Banden beider Kettenteile erklärbar. Vom 13-Ketoester ab ist dann nur mehr die Absorption des Kettenteiles zwischen Ester und Ketogruppe zu beobachten.

Das Frequenzbereich, in dem die Banden mit regelmäßigem Abstand auftreten, variiert bei verschiedenen Substanzgruppen [10]. Im Fall der Ketoester finden wir eine solche Variation bereits innerhalb einer Reihe von Stellungsisomeren, da für die Glieder mit langer Teilkette zwischen Ester- und Ketogruppe die konstant liegenden Banden bei 1380 und 1170 cm^{-1} eine natürliche Grenze bilden, während die regelmäßig angeordneten Banden beim 2-Ketoester zwischen 1340 und 1190 cm^{-1} liegen. Es ist bekannt, daß die Lage der Banden in der Bandenfolge in verschiedenen polymorphen Formen von Säuren verschieden und somit von der Kristallstruktur abhängig ist [2, 19]. Die Untersuchung polymorpher Methylfettsäuren [13] hat gezeigt, daß nicht nur die Lagen, sondern auch die Intensitäten dieser Banden und die Grenze der ganzen Folge stark zwischen verschiedenen polymorphen Formen variiert, sodaß gleiche Begrenzung und gleicher Intensitätsverlauf nur bei gleichen oder ähnlichen Kristallstrukturen zu erwarten sind. In der Reihe der Ketoester unterscheiden sich die „long spacings" des 2-Ketoesters stark von denen der längeren Glieder dieser Reihe [11].

So sind auch die im folgenden beschriebenen, oft beobachteten zwei Typen der Bandenfolgen mit gewissen, noch nicht bekannten, Zügen der Kristallstrukturen verknüpft. Wir finden nähmlich beide Typen bei zwei polymorphen Formen ein und derselben Säure ((−)-3L, 6D-Dimethyltetrakosansäure Form E und F) [13]. Bei dem einen Typ ist keine starke Karboxylbande, die gewöhnlich in diesem Bereich liegt, vorhanden, dafür sind aber alle regelmäßig angeordneten Banden gleichmäßig oder alternierend stark (Typ I, Abb. 3). Bei dem anderen Typ ist eine starke Karboxylbande da; die regelmäßig angeordneten Banden sind wesentlich schwächer und können der starken Bande überlagert sein (Typ II, Abb. 3). Beispiele für Typ I sind die „langkettigen" Ketoester, die von Susi beschriebenen Oktadecensäuren [10], beide polymorphen Formen der 14-Methylheptadekansäure [13], die stabile Form der 12-

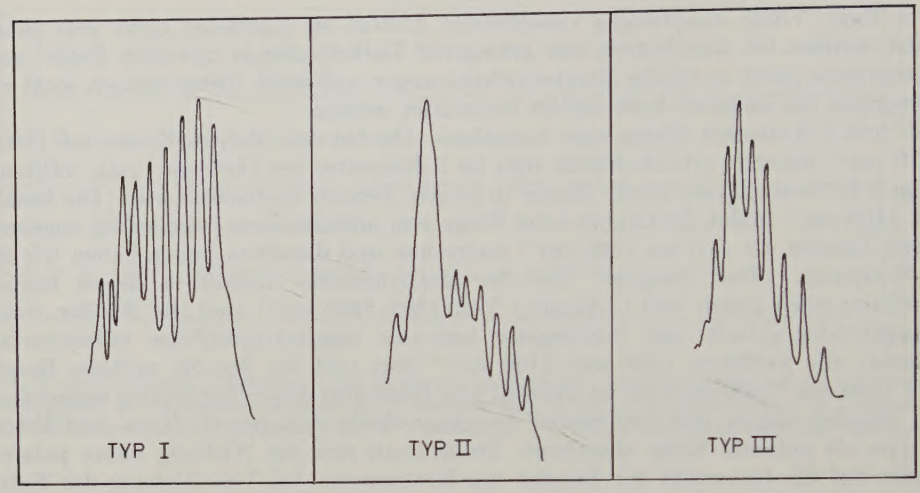

Abb. 3. Häufig auftretende Typen von Bandenfolgen.

Methyltetrakosansäure (13) die 14-Methyloktadekansäure [17] und die Isostearinsäure die als Beispiel abgebildet ist. Typ II ist representiert durch die C-Formen der Palmitin- und Stearinsäure (19), die geradzahligen kürzeren Fettsäuren (C_6 bis C_{12}) (20)[1], beide polymorphen Formen der 33-Methyltetratriakontansäure [12] und die höherschmelzende Form der (−)-3D-Methylnonadekan-(abgebildet) und (−)-3D-Methylheptakosansäure [13].

Ein dritter Typ tritt auf bei allen von uns untersuchten, in 2-Stellung monosubstituierten Fettsäuren und Estern (zehn Stück) (17, 21), sowie auch bei 2-Bromoktadekansäure (3). Er ist gekennzeichnet durch eine regelmäßige Bandenanordnung mit gleichmäßig steigender und wieder fallender Intensität (Typ III, Abb. 3, 2-Ketostearinsäure-methylester). Die stärkste Bande liegt bei Säuren bei 1240 cm^{-1} und bei Estern bei 1290 cm^{-1}. Diese Anordnung ist für die hochschmelzende Form der 2D-Methyloktadekansäure charakteristisch, während die tiefschmelzende Form andere Bandenlagen hat [13]. Auch hier ist also die Bandenfolge bestimmt von der Kristallform und man kann annehmen, daß die in 2-Stellung substituierten Säuren mit ähnlichen Bandefolgen einen gemeinsamen Zug in der Struktur haben.

1150–880 cm^{-1}-Bereich

Zwischen starken Banden bei 1130–1100 cm^{-1} und 890–880 cm^{-1} treten unregelmäßig angeordnete schwächere Banden der C–C-Valenzschwingungen auf. Ihre Lagen sind sogar in den Spektren der Ketoester mit ähnlichen Kristallformen (C = O-Gruppe am C-Atom 6 bis inklusive 13) verschieden und deuten somit auch auf einen Einfluß der Ketogruppe in ihren verschiedenen Stellungen auf die ν(C–C)-Schwingungen hin. Es läßt sich aber keine Gesetzmäßigkeit wie für die wagging- und twisting-Banden feststellen.

[1] Meiklejohn und Mitarbeiter [1] dürften die Dekansäure in einer anderen Kristallform untersucht haben als Corish und Chapman [20], da sich die Spektren um 1200 cm^{-1} so stark unterscheiden.

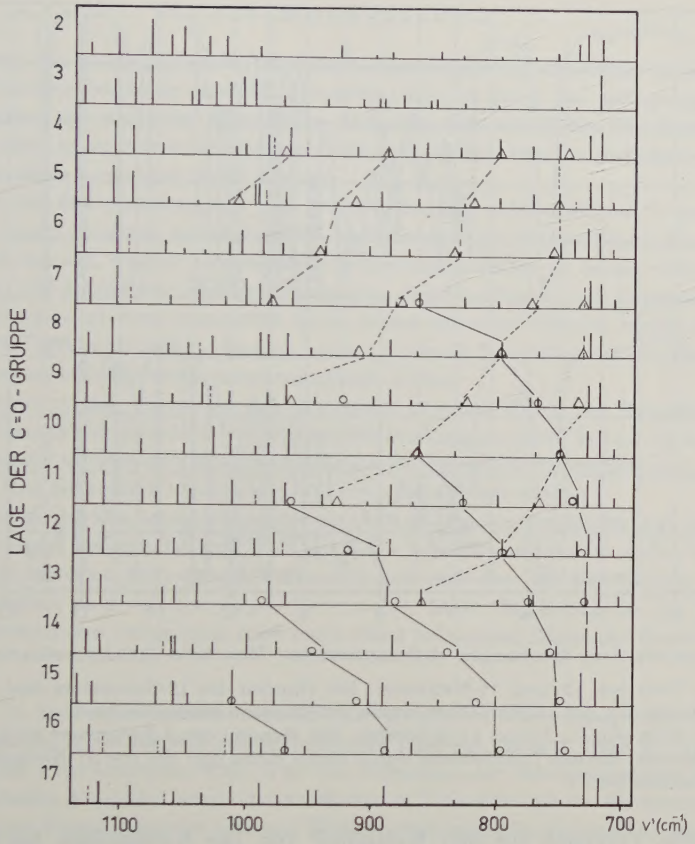

Abb. 4. Schematische Darstellung der Monoketo-oktadekansäuremethylesterspektren von 1140–700 cm^{-1}. Die Strichlänge entspricht der Stärke der Banden.

Bereich der CH_2-rocking-Schwingungen (880–700 cm^{-1})

In den Spektren der Kohlenwasserstoffe [5, 18], der α,ω-Dikarbonsäuremethylester [8] und der kürzeren Fettsäuren (20) wurden Bandenfolgen mit der langwelligen Grenze bei 720 cm^{-1} beobachtet. In den vorliegenden Spektren ist diese Grenze durch ein Dublett bei 729 und 719 cm^{-1}, das durch ein oder zwei Schultern zu einer komplexen Bande wird, gegeben. Nur beim 3-Ketoester ist diese Bande einfach (719 cm^{-1}).

Auch die Banden von 880 bis 720 cm^{-1} liegen für alle Ketoester verschieden und ein Vergleich mit den Spektren der Kohlenwasserstoffe verschiedener Kettenlänge läßt folgende Übereinstimmung finden: Die Ketoester mit n CH_2-Gliedern zwischen Ester- und Ketogruppe haben Absorptionsbanden bei nahezu denselben Wellenzahlen wie die Kohlenwasserstoffe mit n C-Atomen insgesamt. In der schematischen Darstellung der Spektren von 1140–700 cm^{-1} (Abb. 4) sind die in den Ketoesterspektren zu Serien zusammenfaßbaren Banden durch Kurven verbunden und die Lage der bei den Kohlenwasserstoffen auftretenden Banden durch Ringe eingezeichnet.

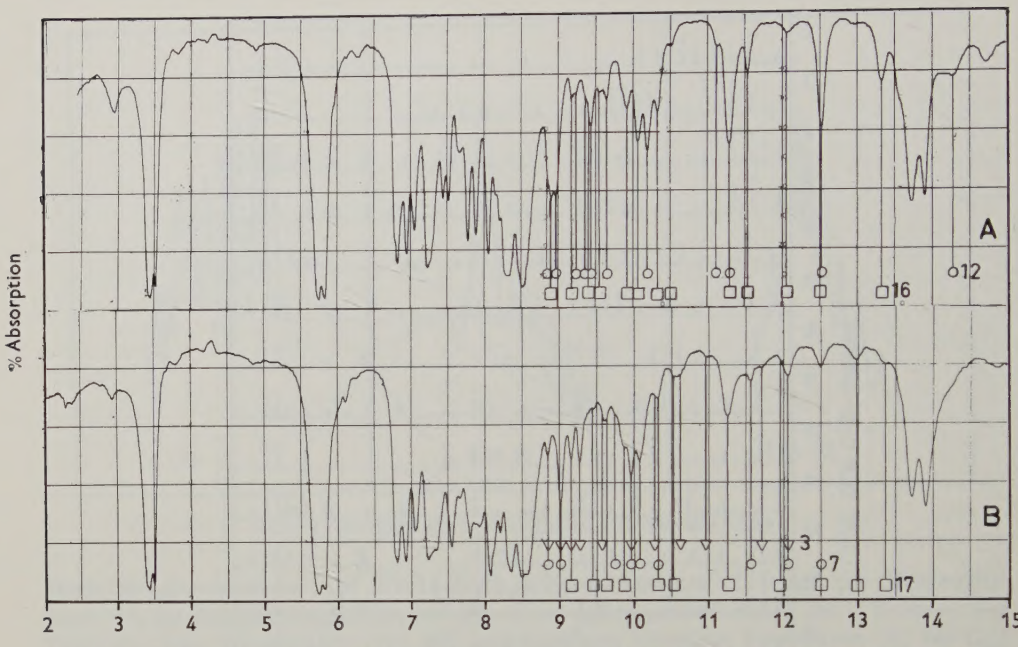

Abb. 5. Spektren von Mischungen stellungsisomerer Monoketo-oktadekansäuremethylester.

A. Gleiche Teile von 12- und 16-Ketoester. Die Banden des 12-Ketoesters sind ab 1140 cm^{-1} durch einen Kreis, die des 16-Ketoesters durch ein Quadrat gekennzeichnet.

B. Gleiche Teile von 3-, 7- und 17-Ketoester. Die Banden des 3-Ketoesters sind ab 1140 cm^{-1} durch ein Dreieck, die des 7-Ketoesters durch einen Kreis und die des 17-Ketoesters durch ein Quadrat gekennzeichnet.

Wird derselbe Vergleich für den Kettenteil von der Ketogruppe bis zur Methylgruppe inklusive durchgeführt, so lassen sich auch hier bei den Bandenlagen der Kohlenwasserstoffsspektren schwache Banden erkennen. Die gestrichelten Kurven in Abb. 4 verbinden diese Banden und die Dreiecke geben die Bandenlagen in den Kohlenwasserstoffspektren an. Daß in diesem Gebiet Banden beider Kettenteile aufzufinden sind wird verständlich, wenn man bedenkt, daß die Intensitäten der rocking-Banden nicht so stark von den Endgruppen abhängig sind wie die der wagging- und twisting-Banden (Abb. 2).

Zur Identifikation von Monoketo-oktadekansäuremethylestern mit unbekannter Karbonylstellung sind die Spektren wegen der in den verschiedenen Spektralbereichen auftretenden Unterschiede außerordentlich gut geeignet. Sogar in Mischungen von zwei oder drei Isomeren können die einzelnen Komponenten erkannt werden, wenn die Bandenlagen in den Mischungsspektren mit denen in den Referenzspektren verglichen werden. (Mischen durch gemeinsames Lösen in Petroleumäther). Dabei zeigt sich, daß die Spektren der einzelnen Isomeren überlagert sind, da alle Banden der Referenzspektren wiederzufinden sind, während keine neuen auftreten. Abb. 5 zeigt zwei solche Spektren, bei denen angegeben ist, in welchem Referenzspektrum die betreffende Bande auftritt. Ein Teil der Banden kommt in zwei oder allen drei Referenzspektren vor, aber es sind immer genügend Banden da, die nur für ein Isomeres charakteristisch sind.

ZUSAMMENFASSUNG

Die Spektren der stellungsisomeren Monoketo-oktadekansäuremethylester werden durch die Lage der Ketogruppe bestimmt. Die Methylenkette zwischen Ester- und Ketogruppe absorbiert wesentlich stärker als der an der Ketogruppe hängende Rest der Kette. Die Ketogruppe teilt somit das Molekül absorptionsmäßig in zwei Teile und die Spektren der Stellungsisomeren sind dadurch denen einer homologen Reihe ähnlich.

Im Bereich der Methylen-wagging- und -twisting-Banden (1380–1150 cm^{-1}) treten bei den Gliedern mit langer Teilkette zwischen Ester- und Ketogruppe Bandenfolgen mit regelmäßigen Frequenzabständen auf, die sich in der Reihe der Stellungsisomeren zu Serien verbinden lassen, ähnlich wie bei den homologen normalen Fettsäuren. Bei den Gliedern mit längerem Kettenrest an der Ketogruppe sind diese, vermutlich durch dessen störenden Einfluß, häufig aufgespalten.

Im Bereich der Methylen-rocking-Banden können für beide Kettenteile Serien von Banden wie in den Kohlenwasserstoffspektren zusammengefaßt werden.

Das Vorherrschen der Absorption des Kettenteils zwischen Ester- und Ketogruppe bedingt so deutliche Unterschiede zwischen den Spektren der Stellungsisomeren, daß sie zur Identifikation von Ketoestern mit unbekannter Karbonylstellung außerordentlich gut geeignet sind. Mischungen von zwei oder drei Ketoestern können ebenfalls noch identifiziert werden.

Die Bandenfolge der hier behandelten Ester und einer Reihe von Fettsäuren lässt sich, nach der Intensitätsverteilung der Banden und nach der Lage ihrer Begrenzung, in vielen Fällen einem von drei Typen zuordnen. Polymorphe Umwandlungen verändern die Bandenfolge und können Übergänge zwischen zwei der drei Typen hervorrufen. Diese Charakteristika der Bandenfolge sind also mit bestimmten (wenn auch noch nicht näher bekannten) Zügen der Kristallstrukturen verknüpft.

Herrn Prof. E. Stenhagen möchte ich für die Anregung zur Untersuchung substituierter langkettiger Fettsäuren, sowie für sein stetes förderndes Interesse danken. Frau K. Nilsson danke ich für wertvolle experimentelle Hilfe. Für die Aufnahme der Röntgenspektren sei Herrn A. Westerdahl gedankt. Finanziell wurde die Arbeit durch Unterstützung des Schwedischen Naturwissenschaftlichen Forschungsrates (Statens Naturvetenskapliga Forskningsråd) ermöglicht.

Die Arbeit wurde an der Abteilung für Medizinische Biochemie am Medizinisch-Chemischen Institut der Universität Uppsala, Uppsala, Schweden, durchgeführt.

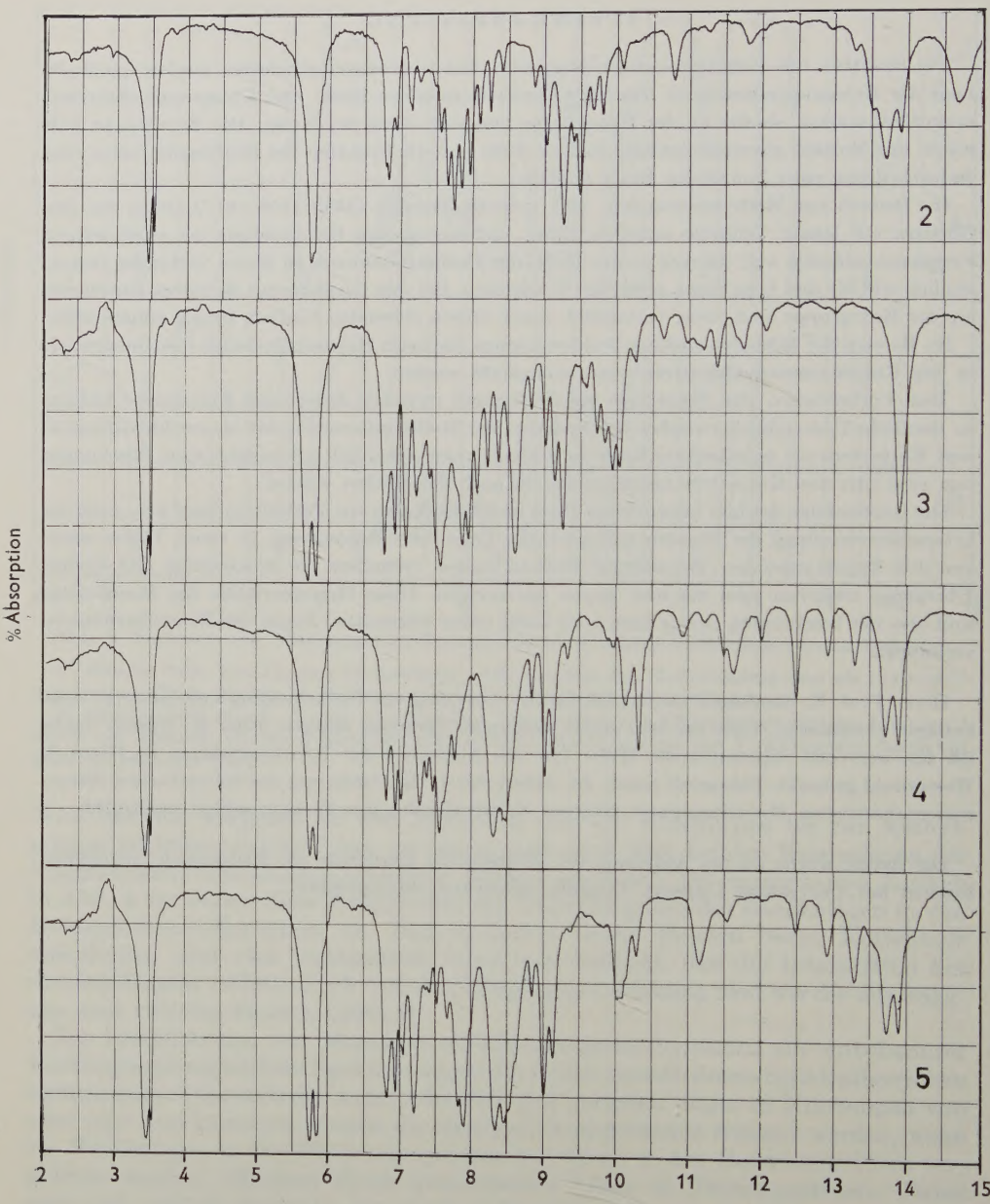

Abb. 6. Spektren des 2-, 3-, 4- und 5-Keto-oktadekansäuremethylesters.

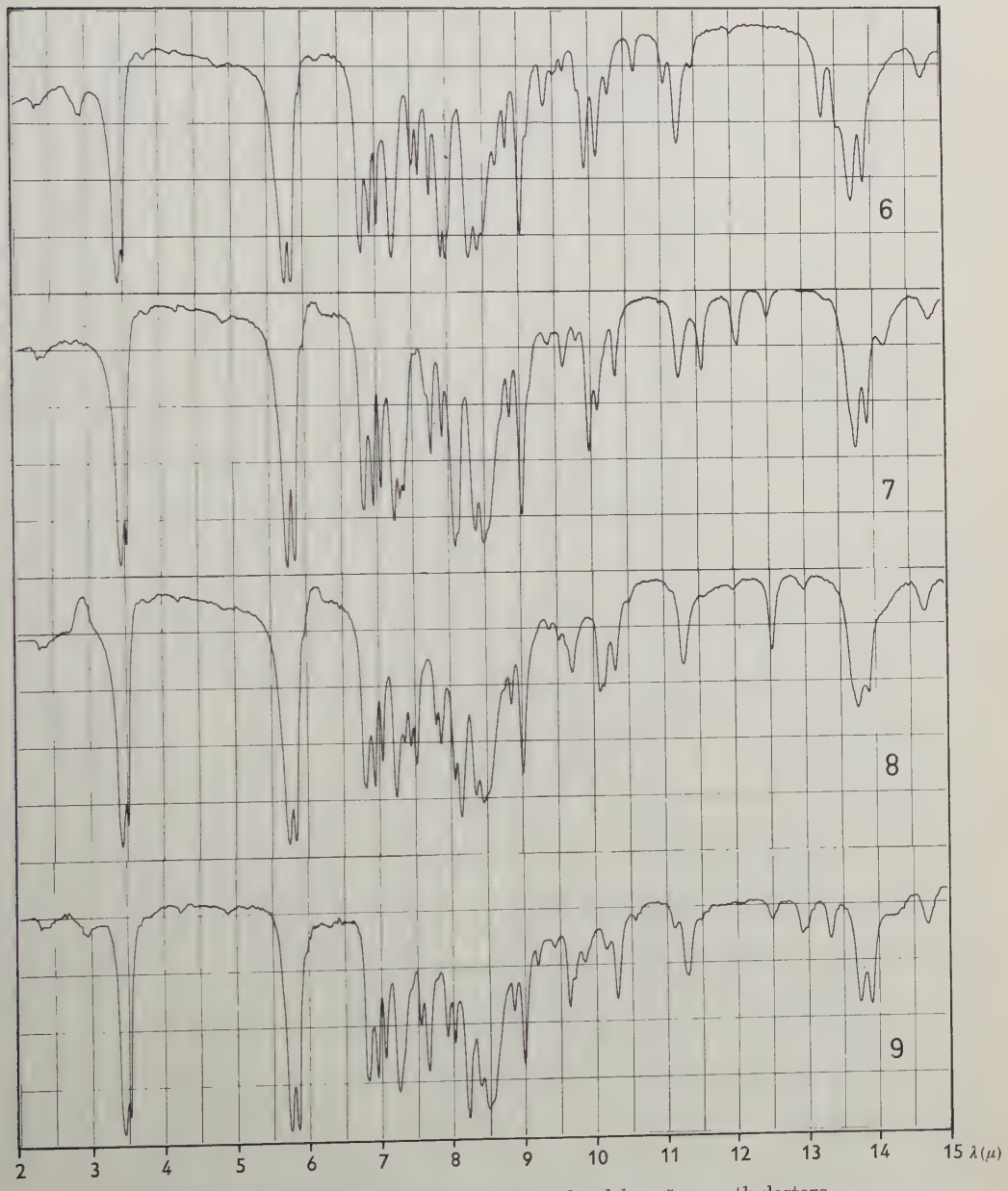

Abb. 7. Spektren des 6-, 7-, 8- und 9-Keto-oktadekansäuremethylesters.

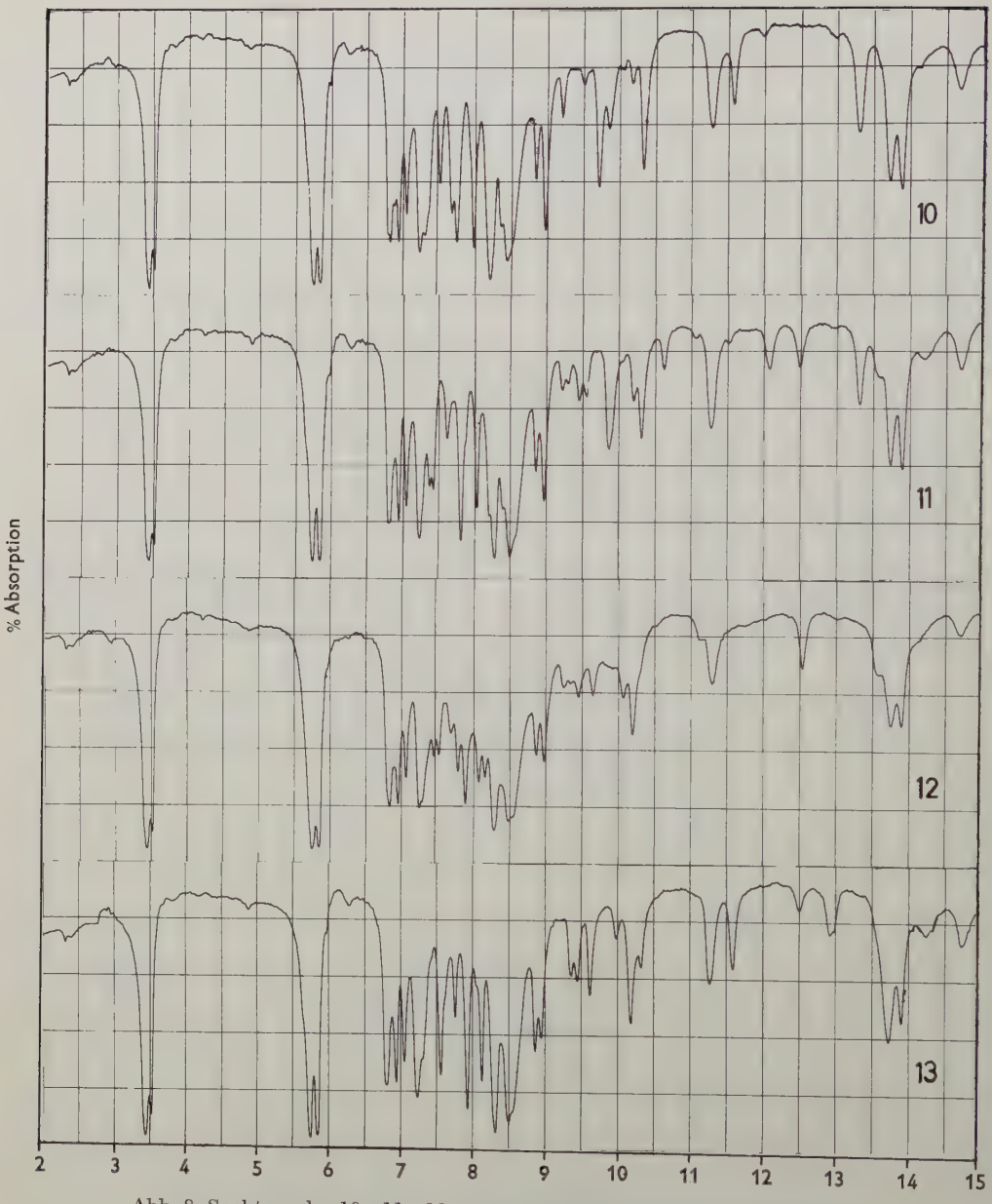

Abb. 8. Spektren des 10-, 11-, 12- und 13-Keto-oktadekansäuremethylesters.

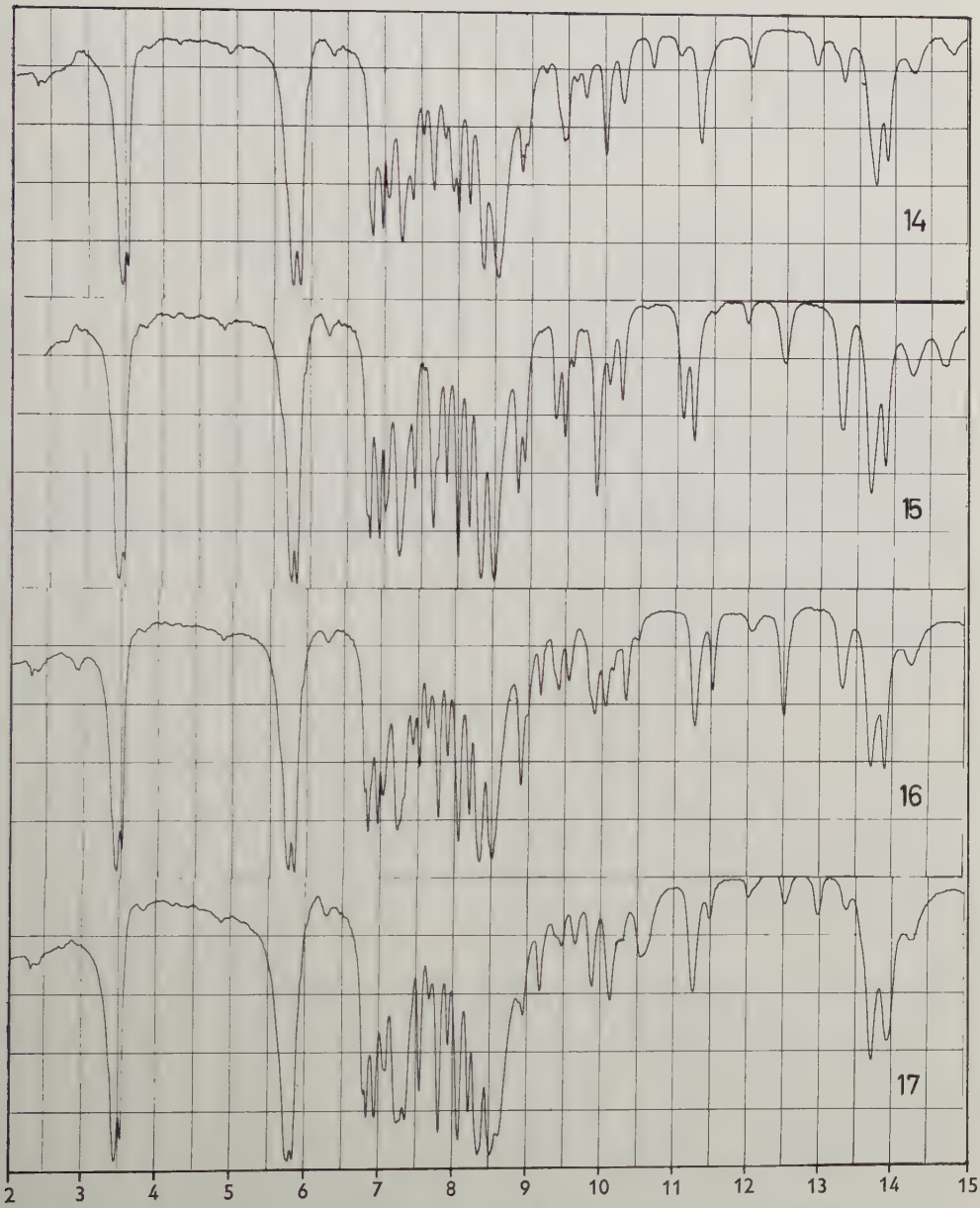

Abb. 9. Spektren des 14-, 15-, 16- und 17-Keto-oktadekansäuremethylesters.

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Tryckt den 21 september 1961

Uppsala 1961. Almqvist & Wiksells Boktryckeri AB

ARKIV FÖR KEMI Band 18 nr 5

Mitgeteilt am 7. Juni 1961 durch Arne Fredga

Über 1-acetyl-5-pyrazolon-3-carbonsäureäthylester

Von Erik Wahlberg

In einer früheren Mitteilung wurde die Vermutung ausgesprochen, dass die bei der Darstellung von Oxalessigsäureäthylester-*N*-acetylhydrazon als Nebenprodukt angegebene Substanz 1-Acetyl-5-pyrazolon-3-carbonsäureäthylester wäre [1]. Die nachträgliche Untersuchung bestätigte wohl, dass ein grosser Teil des Produktes aus dieser Substanz bestand; beim Umkristallisieren wurden aber nur Zersetzungsprodukte wie 5-Pyrazolon-3-carbonsäureäthylester und etwas Diacetylhydrazin isoliert.

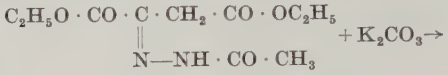
Es wurde nun versucht, das gewünschte Pyrazolon aus Oxalessigester-*N*-acetylhydrazon darzustellen. Wie zu erwarten war, zeigte es sich, dass die Acetylgruppe durch Natriumäthylat in Alkohol-Ätherlösung abgespalten wurde. Auch das Kochen in verschiedenen Lösungsmitteln hat teilweise denselben Erfolg.

Beim Versuch, die NH_4-Verbindung des gewünschten Pyrazolons auf dieselbe Weise wie beim *N'*-carbäthoxyhydrazon [2] darzustellen, wurde nur ein Gemisch mit 37 % der gewünschten Substanz mit der NH_4-Verbindung des Zersetzungsprodukts Pyrazoloncarbonsäureäthylester erhalten. Die Mischung hatte den Schmelzpunkt 146–147°. Nach dem Umkristallisieren wurde freilich dieser auf 149–150° und der Gehalt auf 50 % erhöht. Eine reine Substanz konnte nicht auf diese Weise erhalten werden. Übrigens gab die Mischung mit Essigsäure behandelt, nur Pyrazoloncarbonsäureester.

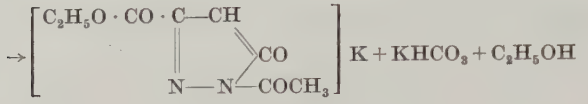
Es wurde nun die Behandlung von Äther- oder Bensollösungen mit wässrigen Lösungen von Na_2CO_3, $NaHCO_3$ und Na_2HPO_4 versucht. Analysen zeigten, dass auf diese Weise nur ein Gehalt von etwa einem Drittel des Acetylderivates erhalten werden konnte. Die alkoholische Lösung der Kristalle gab mit $FeCl_3$ eine braunviolette Farbe, welche doch ziemlich schnell heller wurde und sich zum Schluss der Farbe des Pyrazoloncarbonsäureesters näherte. Als eine mit Uhrglas lückenhaft bedeckte, vorher getrocknete Bensollösung des erwähnten Reaktionsproduktes bei Zimmertemperatur sehr langsam (eine Woche) verdunstet wurde, konnte ein unverkennbaren Geruch von Essigsäure beobachtet werden. Der Rückstand bestand nahezu allein aus dem Zersetzungsprodukt Pyrazoloncarbonsäureester. Es geht daraus deutlich hervor, dass die Luftfeuchtigkeit die Zersetzung herbeigeführt hatte.

1-Acetyl-5-pyrazolon-3-carbonsäureäthylester wurde darum auf die folgende Weise isoliert: Oxalessigsäureäthylester-*N*-acetylhydrazon (I) wurde in trocknem Aceton

gelöst und mit getrocknetem Kaliumcarbonat geschüttelt. Dabei wurde Äthanol abgespalten und die in Aceton völlig unlösliche Kaliumverbindung (II) des Acetylpyrazoloncarbonsäureäthylesters als sehr feinen Schlamm abgeschieden.



I



II

Die Kaliumverbindung gab nach Behandlung mit einer Lösung von Phosphorsäure in trockenem Äther nahezu völlig reinen Acetylpyrazoloncarbonsäureäthylester als eine weisse Masse.

Die Verbindung ist in gewöhnlichen Lösungsmitteln leicht löslich. Bei dem Versuch der Umkristallisation kristallisiert der schwerer lösliche Pyrazoloncarbonsäureäthylester aus. Er löst sich in Sodalösung ohne Färbung und unter teilweiser Zersetzung und gibt mit äthanolischer $FeCl_3$-Lösung eine dunkle, braunviolette Farbe, die ziemlich schnell aufhellt und der Farbe des Pyrazoloncarbonsäureäthylesters ähnlich wird. Die Substanz hat keinen Schmelzpunkt. Sie sintert allmählich, und die Schmelze wird erst gegen 175° klar, also bei dem Schmelzpunkt des Pyrazoloncarbonsäureäthylesters (179–185°). Sie ist sehr hygroskopisch und sintert schon bei dem Abwiegen für die Analyse.

Versuche

2,44 g (0,01 mol) Oxalessigesteracetylhydrazon wurden in 200 ml über K_2CO_3 getrocknetem Aceton gelöst und mit 1,38 g (0,01 Mol) sehr fein pulverisiertem, getrocknetem K_2CO_3 in einer gut geschlossenen Flasche 16 St. geschüttelt. Der so erhaltene Schlamm wurde schnell abgesaugt, mit über K_2CO_3 getrocknetem Aceton mehrmals gewaschen und im Vakuum getrocknet. Bei der Analyse wurden von 0,1795 g Substanz 0,0892 g K_2SO_4 erhalten, was einem Gehalt von 22,34 % K entspricht. Kaliumkarbonat (K_2CO_3) konnte also in der Mischung nicht übrig sein, denn bei der Annahme der alleinigen Anwesenheit von $KHCO_3$ wurde ein Gehalt von 23,24 % K berechnet. Die Mischung enthielt N 8,51%. Ber. 8,64%.

1-Acetyl-5-pyrazolon-3-carbonsäureäthylester wurde aus der erwähnten Mischung auf folgender Weise erhalten:

1,24 g der Mischung wurde mit 20 ml 0,1-*M* H_3PO_4 in trocknem Äther eine halbe Stunde geschüttelt und danach abgesaugt. Die Ätherlösung wurde im Vakuum abgedampft. Es wurde ein weisses, sehr hygroskopisches Pulver erhalten. Beim Stehen in einem offenem Gefäss sintert das Pulver zusammen unter Abgabe von Essigsäure.

$C_8H_{10}O_4N_2$ (198,18)	Ber.	C 48,48	H 5,09	N 14,14
	Gef.	C 48,32	H 5,14	N 13,93

Professor Arne Fredga, der Arbeitsplatz und Ausrüstung zur Verfügung stellte, bin ich zu grossem Dank verpflichtet. Auch dem *Schwedischen Naturwissenschaftlichen Forschungsrat* und *A.S.E.A.*, Västerås bin ich für ihre Unterstützung sehr dankbar.

Chemisches Institut der Universität Uppsala.

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Tryckt den 21 september 1961

Uppsala 1961. Almqvist & Wiksells Boktryckeri AB

ARKIV FÖR KEMI Band 18 nr 6

Communicated 8 March 1961 by Karl Myrbäck and Arne Fredga

On the transformations of unstable thienyllithium compounds

By Pinchas Moses[1] and Salo Gronowitz

When an organic compound containing hydrogens of various "acidities" is metalated with organolithium compounds [1], the most positively polarised (acidic) hydrogen will usually react most rapidly, directly giving the thermodynamically most stable lithium compound. With aromatic compounds containing acidic hydrogens as well as halogens, capable of undergoing halogen–metal interconversion [2], not situated at the most acidic position, the situation is more complicated. In such cases, three main types of reactions have been observed to occur, as demonstrated by the following examples.

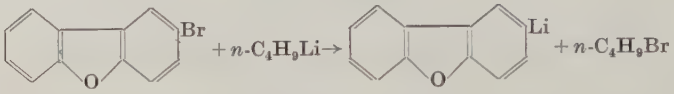
When 2-bromodibenzofuran was treated with excess *n*-butyllithium, halogen–metal interconversion occurred and 87 % of 2-dibenzofurancarboxylic acid was obtained upon carbonation after 30 minutes [3]. Similarly, Gronowitz [4] obtained 3-thiophenecarboxylic acid upon carbonating the product of reaction between *n*-butyllithium and 3-bromothiophene at −70°, whilst Lawesson obtained 4-bromo-2-thiophenecarboxylic acid [5] and 4-bromo-3-thiophenecarboxylic acid [6] by treating 2,4- and 3,4-dibromothiophene respectively with *n*-butyllithium at −70° and carbonating the reaction mixture after short periods.

If the reaction between the organolithium compound and the aromatic halide is allowed to proceed for longer periods and at higher temperatures, metalation of the halide rather than interconversion often occurs. Gilman *et al.* [7] obtained 2-bromo-4-dibenzofurancarboxylic acid in 24 % yield by refluxing an equimolar mixture of *n*-butyllithium and 2-bromodibenzofuran for 9 hours in benzene before carbonating the mixture, Gronowitz [4] obtained 3-bromo-2-thiophenecarboxylic acid by reacting *n*-butyllithium with 3-bromothiophene at room temperature and Lawesson obtained 2,4-dibromo-5-thiophenecarboxylic acid [5] and 3,4-dibromo-2-thiophenecarboxylic acid [6] by reacting the appropriate reagents also at room temperature. The influence of the time factor on the course of this type of reaction is convincingly demonstrated by Gilman's experiments with *p*-iodoanisole [3]. Gilman *et al.* reacted *p*-iodoanisole with *n*-butyllithium and carbonated aliquots of the reaction mixture after various time intervals; after 1 hour *p*-methoxybenzoic acid only was obtained, whilst after 10 hours only 2-methoxy-5-iodobenzoic acid was isolated. In the same paper, these authors demonstrated the influence of the proportion of reactants on the course of reaction. When *p*-bromoanisole was reacted with a 200 % excess of *n*-butyllithium, only halogen–metal interconversion occurred and *p*-methoxybenzoic acid was iso-

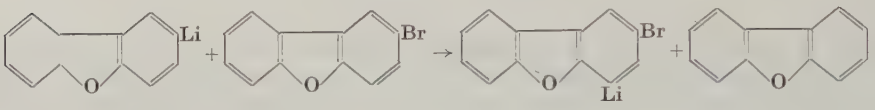
[1] On leave of absence from the Hebrew University of Jerusalem, Israel.

lated exclusively upon carbonation, whilst only metalation occurred if the halide was in 200 % excess, giving 47–52 % yield of 2-methoxy-5-bromobenzoic acid; equimolar quantities of reactants yielded mixtures of *p*-methoxybenzoic acid and 2-methoxy-5-bromobenzoic acid.

The above-mentioned authors are of the opinion that the metalation takes place by a two-stage mechanism, and is preceded by halogen–metal interconversion. Thus Gilman *et al.* [3] proposed the following scheme for the metalation of 2-bromodibenzofuran by *n*-butyllithium:

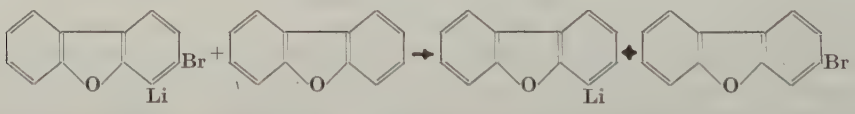


(1)



(2)

Besides halogen–metal interconversion and metalation, the reaction between *n*-butyllithium and aromatic halides has been known in a few cases to lead to rearrangement. Gilman and Melstrom [8] have found that when 3-bromobenzofuran was reacted with excess *n*-butyllithium at −70° and subsequently carbonated, 3-benzofurancarboxylic acid was obtained; longer reaction times and higher temperatures led to the isomeric 2-acid. The low yields (ca. 12 %) were due to ring opening to give *o*-hydroxyphenyl acetylene. Similarly, when Gilman *et al.* [9] refluxed 3-bromodibenzofuran with *n*-butyllithium, they obtained a mixture of 3- and 4-dibenzofurancarboxylic acid after carbonation, the latter in excess. These authors proposed an intermolecular rearrangement mechanism (eq. 3) following an initial 2-stage metalation reaction (cf. eq. 1 & 2):



(3)

We thus see that *n*-butyllithium can react with aromatic halides in diverse ways, the kind of reaction actually taking place depending upon various factors, such as reaction temperature, reaction time, proportions of reactants, mode and rate of addition, local concentration and heating, etc.

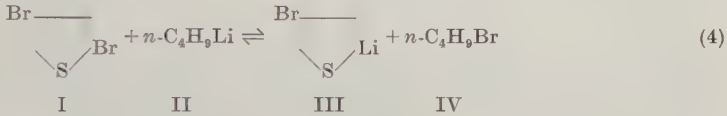
The halogen–metal interconversion reaction (at −70°) with substituted bromothiophenes, where the bromine is not situated in the most acidic position, has been extensively used by the present authors for the preparation of various substituted thiophenes [10, 11, 12]. However, it was noted on some occasions that unexpected reaction products were obtained [10]. This anomalous behaviour in the reaction between *n*-butyllithium and some bromothiophenes, and the resulting uncertainty as to the preparative value of this method, led the present authors to investigate in greater detail this type of reaction. 3-Bromothiophene and 2,3- and 3,4-dibromothiophene have been studied briefly, whilst 2,4-dibromothiophene has been chosen for a more detailed investigation. The above-mentioned isomeric *ortho* dibromothiophenes are of especial interest in connection with the current investigations of the

dehydrobenzene (benzyne) problem (for review, cf. ref. 13). The remarkable stability of the *ortho* bromothienyllithium as compared with the instability of the analogous *ortho* halophenyllithium has already been pointed out by us [10], and it might as well be mentioned that, hitherto, no evidence has been forthcoming to indicate the existence of a transient dehydrothiophene.

Reactions with 2,4-dibromothiophene

2,4-Dibromothiophene was reacted with *n*-butyllithium under various experimental conditions.

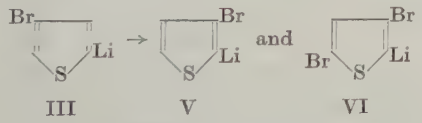
Confirming Lawesson's finding [5], we obtained 4-bromo-2-thiophenecarboxylic acid upon carbonation of the product of reaction between *n*-butyllithium and the halide at −70°.



(4)

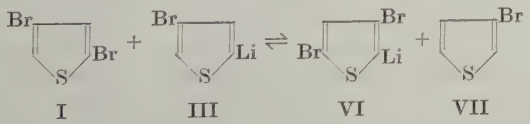
High yields can be obtained in this reaction (Table 1, exp. 3 I and 3 II) showing that the equilibrium position in eq. 4 lies far to the right. The bromothienyllithium III is fairly stable at −70°, and only about 10 % of it underwent further transformations while standing at the low temperature for 12 hours (Table 1, exp. 1 II and 2 II).

If the reaction mixture is allowed to come up to room temperature after the 4-bromo-2-thienyllithium III had been formed at −70°, there is a general tendency for III to disappear with the simultaneous appearance of a mixture of 3-bromo-2-thienyllithium V and 2,4-dibromo-5-thienyllithium VI, in varying proportions (Table 1, exp. 3 III, 4 III, 5, 6, 7, 8).

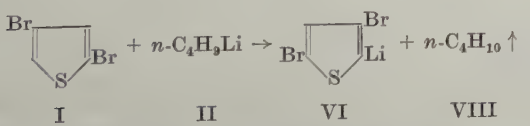


(5)

The possible routes whereby VI might be formed are described by eq. 6 and 7,



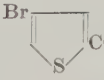
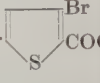
(6)



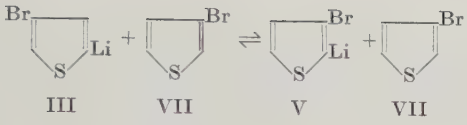
(7)

whilst the 3-bromo-2-thienyllithium may be formed according to eq. 8 and 9 or, much less probably, by route 10 or by an intramolecular rearrangement mechanism:

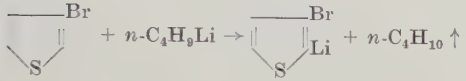
Table 1. Reaction between n-C_4H_9Li and .

No. of exp.	Aliquot no.	Moles of halide	Final conc.[a] of halide	Time, −70°	Time, room temp.	% composition and yields of product mixture[b] 		
1	I	0.05	0.33	10 min		100(51)		
	II			12 h		87(49)		13(5)
2	I	0.05	0.31	10 min		100(56)		
	II			11 h		91(54)		9(4)
3	I	0.075	0.43	5 min		100(81)		
	II			1 h		100(81)		
	III				17 h		36(28)	64(36)
4	I	0.05	0.33	5 min		100(67)		
	II			1 h 30 min		100(67)		
	III				19 h		49(48)	51(36)
5		0.20	0.68	5 min	16 h		73(65)	27(17)
6	I	0.04	0.50	5 min	1 h 50 min	88		12
	II				5 h 15 min	40	40	20
7[c]	I	0.044	0.55	5 min	1 h 50 min	67	8	25
	II				5 h 5 min	34	41	25
8[d]	I	0.044	0.49	5 min	1 h 45 min	54	23	23
	II				5 h	18	61	21
9		0.05	0.33		2 h 30 min	20(12)		80(35)
10		0.04	0.38		4 h		5(3)	95(40)
11		0.025	0.36		5 min	71		29
12		0.03	0.50		5 min	58(41)		42(21)
13[e]		0.03	0.50		5 min	65(40)	9(6)	26(12)
14		0.20	0.71		5 h	42(30)	7(5)	51(27)
15[e]		0.20	0.61		10 min	38(28)	31(23)	31(17)
16	I	0.15	0.52	5 min	50 min	100(65)		
	II				2 h 30 min	100(77)		
	III				3 h 45 min	100(68)		
	IV				8 h 30 min	100		
	V				17 h	88(68)	12(10)	
	VI				27 h 30 min	64(42)	36(23)	
	VII				69 h 45 min	7(4)	93(58)	
17	I	0.15	0.52		1 h	64(43)		36(17)
	II				2 h 45 min	63(30)		37(13)
	III				4 h	62(37)		38(16)
	IV				8 h 45 min	63(33)		37(14)
	V				17 h 30 min	50(31)	10(6)	40(18)
	VI				27 h 45 min	43(25)	14(8)	43(18)
	VII				70 h	7(4)	51(30)	42(18)

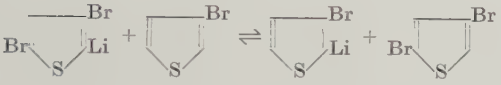
[a] Moles/liter (after mixing the reactants).
[b] Yields in parenthesis.
[c] Equimolar quantities of halide and n-butyl lithium.
[d] Equimolar quantities of halide and n-butyl lithium; 10 % of 3-bromothiophene added to reaction mixture after removal of cooling bath.
[e] "Reverse" addition.



III VII V VII (8)



VII II V VIII (9)



VI VII V I (10)

It should be pointed out that, after equilibrium conditions have been obtained at −70° according to eq. 4, and the temperature is allowed to rise, all or any of the secondary reactions 6–10 may proceed simultaneously—or nearly so—at various rates, leading to the transformation 5.

Upon examination of the possible processes whereby transformation 5 could be brought about, certain general features immediately become apparent:—

Large quantities of the metalated product VI could, in principle, be formed even if only a very small amount of I is present at the equilibrium state of 4, since, with the removal of I by process 6 or 7, the equilibrium in 4 will be continually displaced to the left. The amount of metalated product VI formed after a given time will depend, to a first approximation, on the residual concentration of I after the primary reaction 4 has come to equilibrium conditions. If a relatively large excess of *n*-butyllithium was used, the residual concentration of I will be greatly reduced and VI will be formed very slowly. Conversely, if there is a relative deficiency of *n*-butyllithium, the residual concentration of I will be greater and VI will be formed much more rapidly (cf. ref. 3).

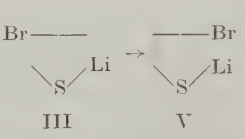
Since VI is the thermodynamically most stable metalated bromothiophene, it may be considered, as a first approximation, not to undergo further transformations once it has been formed; hence process 10 may carry only insignificant weight.

3-Bromothiophene seems to be an indispensable link in the rearrangement reaction; if this proceeds primarily by route 8 (and the evidence favouring this route will be presented later), it is evidently possible for all of III to be completely converted to V in the presence of only catalytic amounts of 3-bromothiophene.

In view of the above considerations, it is not surprising that the percentage composition of the products resulting from transformation 5 will vary greatly in different experiments, since small and difficultly controllable differences in the experimental conditions (such as the excess of *n*-butyllithium used; accidental hydrolysis of traces of III to give catalytic amounts of VII) will cause great differences in the final proportions of V to VI. Thus, referring to Table 1, exp. 3 III, 4 III and 5, we see that the product mixtures contain 64 %, 51 % and 27 % of metalated product VI respectively, the balance consisting of rearranged product V, whilst in exp. 16 VII, 93 % of rearranged product appears in the product mixture without the accompanying appearance of even detectable amounts of VI by IR-spectroscopy.

As has been mentioned already, the metalated product VI could be formed by two routes, 6 and 7. Although Gilman seems to give preference to a 2-stage metalation mechanism (corresponding to route 6) in his studies [3, 7], experimental evidence in the present work indicates that both routes are operative. By reasoning alone, one would favour route 6, since the concentration of III is much greater than that of II when equilibrium conditions have been attained in 4 (usually, a maximum of 10 % excess of *n*-butyllithium was used in these experiments). Experimentally, it is found that the appearance of VI is invariably accompanied by the appearance of the rearranged product V; since formation of the latter apparently necessitates the previous formation of 3-bromothiophene, it seems that at least part of VI is formed by route 6. On the other hand, when we examine the yields of acidic products actually isolated in experiments 3 III, 4 III and 5 (see Table 1), calculated[1] on the assumption that route 6 *only* is operating, we find that they are 100 %, 120 % and 99 % respectively. These impossibly high yields indicate that at least part of VI was formed according to route 7.

With regard to the rearrangement of III to V



III V (11)

all evidence points to route 8 being the main operative cause. Referring to exp. 5 (Table 1) we see that after 16 hours at room temperature, all the III which had initially been formed at −70° was transformed to a mixture consisting of 73 % V and 27 % VI. A yield of 17 % of VI indicates a maximum yield of 17 % 3-bromothiophene. Since, however, a much greater yield (65 %) of 3-bromo-2-thiophenecarboxylic acid was actually isolated, it seems that the rearrangement proceeds by route 8 rather than by route 9, since by the latter one can never get more of V than VI formed. Furthermore, in a subsequent experiment (Table 1, exp. 8), 10 % (molar) of VII was added to the reaction mixture five minutes after equimolar quantities of I and II were mixed at −70°; carbonation of aliquots after various time intervals at room temperature revealed that rearrangement had occurred to a much greater extent than in a corresponding experiment (Table 1, exp. 7), where no VII had been added. This indicates that VII plays a decisive role in the rearrangement, and route 8 is of greater importance than intramolecular rearrangement [9].

When an ethereal solution of 2,4-dibromothiophene is added at room temperature to the *n*-butyllithium, a mixture of III and VI is usually formed during the mixing period which, upon protracted standing, has the tendency to transform to a mixture consisting of various proportions of V and VI (see exp. 17 I–VII, Table 1). This latter type of transformation has already been discussed, and it seems that the picture drawn for the case where the reactants are mixed at −70° and then allowed to come up to room temperature applies here too. With regard to the initial reaction, there are good reasons for supposing that in this case VI is formed predominantly by direct metalation with *n*-butyllithium (route 7) and not by a 2-stage mechanism.

[1] The yields of 2,4-dibromo-5-thiophenecarboxylic acid given in the tables are calculated on the basis of direct metalation (route 7); if route 6 is operative, the figures cited must be multiplied by a factor of two.

Referring to Table 1, exp. 9, we see that 35 % of VI had been formed without the accompanying appearance of detectable amounts of V, 40 % of VI had been formed in exp. 10 with the appearance of only 3 % of V after a reaction time of 4 hours and 27 % of VI had been formed in exp. 14 with the appearance of only 5 % of V after 5 hours. In exp. 17 no V at all has appeared after a reaction time of 9 hours, though considerable amounts of VI had been formed already after 1 hour; similar observations were noted in experiments 11 and 12. It cannot be argued that these results are explainable on the basis of the rearrangement reaction proceeding at a relatively slow rate, for we saw from exp. 8 that when 10 % of 3-bromothiophene was added to the reaction mixture, rearranged product was appearing already after $1\frac{3}{4}$ hours' reaction time.

Further evidence favouring route 7 in these reactions is derived by the examination of the neutral (non-acidic) products obtained in exp. 14. By fractionation of the neutral products obtained in this experiment, 7 % of 3-bromothiophene was isolated; if one adds to this a further 5 % corresponding to the amount of rearranged product formed in this experiment, we obtain an absolute maximum yield of 12 % of 3-bromothiophene, as compared with the 27 % of VI formed. Actually, the amount of 3-bromothiophene formed will certainly be less than 12 %, for the rearranged product is mainly formed by route 8, which does not consume any VII. It thus seems highly probable that the bulk of VI formed in these experiments was derived by direct metalation of I with *n*-butyllithium and not by a 2-stage mechanism, which involves the production of a corresponding quantity of VII. When, however, the "reverse"[1] mode of addition is adopted, i.e. the *n*-butyllithium is added to the bromothiophene, metalation seems to take place via a 2-stage mechanism. In experiments 13 and 15, the *n*-butyllithium was added to an ethereal solution of I; under these conditions, there is plenty of opportunity for reaction 6 to take place at a reasonable rate, and that this had indeed occurred is evidenced by the appearance of rearranged product. In exp. 13, 6 % of V appeared after a reaction time of only 5 minutes and in exp. 15, 23 % of rearranged product appeared after 10 minutes, showing that rearrangement proceeds at a fairly rapid rate. In both these cases, the amount of VI formed was less than that generally formed in the experiments with the "normal" mode of addition, yet in spite of that, rearrangement had occurred here after a short time but not in the above experiments, indicating that in "reverse" addition, VI is formed with production of VII (i.e. route 6) whereas in "normal" addition no VII, or only very little, is produced (i.e. route 7).

Summarising the above discussion, we obtain the following picture with respect to the reaction between *n*-butyllithium and 2,4-dibromothiophene:

If *n*-butyllithium is added to 2,4-dibromothiophene at $-70°$, halogen–metal interconversion occurs and 4-bromo-2-thiophenecarboxylic acid is obtained in high yield after carbonation. The 4-bromo-2-thienyllithium is fairly stable at $-70°$ up to 10 hours. If the reaction mixture obtained at $-70°$ is allowed to come up to room temperature, the 4-bromo-2-thienyllithium will gradually disappear, some 2,4-dibromo-5-thienyllithium will eventually appear—partly as the result of a 2-stage mechanism—giving rise to the formation of 3-bromothiophene and the consequential appearance of 3-bromo-2-thienyllithium. There are some indications that the metalated product is formed by direct metalation with *n*-butyllithium as well. The amount of *n*-butyllithium used will be responsible for the residual concentration of dibromo-

[1] In this work, addition of the bromothiophene to the *n*-butyllithium will be referred to as "normal" addition, whilst the converse mode of addition will be referred to as "reverse" addition.

thiophene, and hence will govern the rate of metalation, especially if this takes place mainly via the 2-stage mechanism. If a relatively large excess of *n*-butyllithium is used, the metalated product will be formed very slowly, hence in this case, the product mixture will contain much more rearranged product than metalated product.

If the halide is added to the *n*-butyllithium at room temperature, halogen–metal interconversion and direct metalation proceed simultaneously, resulting in mixtures of 4-bromo-2-thiophenecarboxylic acid and 2,4-dibromo-5-thiophenecarboxylic acid upon carbonation.

If the *n*-butyllithium is added to the halide at room temperature, halogen–metal interconversion occurs initially together with metalation, the latter taking place mainly by a 2-stage mechanism. As a result, rearrangement occurs fairly rapidly and 3-component mixtures will be isolated if the reaction mixture is carbonated shortly after the reaction is initiated.

Reactions with 3-bromothiophene

This compound is the simplest example of a bromothiophene where the interchangeable halogen is not situated at the most acidic site. Although it may be expected to undergo reactions similar to those observed with 2,4-dibromothiophene, yet the α-hydrogens here may well be less reactive than in the 2,4-dibromothiophene, the latter's α-hydrogen being activated by two bromine atoms.

When the halide is added to *n*-butyllithium at −70°, halogen–metal interconversion occurs and 3-thiophenecarboxylic acid is obtained after carbonation. The 3-thienyllithium is stable at −70° even after 10½ hours (see Table 2, exp. 1, 2 and 3).

Table 2. Reaction between *n*-C_4H_9Li and .

No. of exp.	Aliquot no.	Moles of halide	Final conc.[a] of halide	Time, −70°	Time, room temp.	% composition of product mixture[b]		
1		0.30	0.69	5 min		100(78)		
2		0.05	0.41	5 h 45 min		100		
3		0.05	0.50	10 h 30 min		100		
4	I	0.075	0.50	5 min	1 h	100		
	II				2 h 30 min	95	5	
	III				4 h	95	5	
	IV				24 h	Oil		
5		0.025	0.36		5 min	85(43)	15(5)	
6[c]		0.05	0.49		5 min	95	5	
7[c]		0.05	0.49		5 min	85	15	
8		0.2	0.70	5 min	16 h		44(15)	56(29)

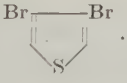
[a] Moles/liter (after mixing the reactants).
[b] Yields in parenthesis.
[c] Oil was obtained when a second aliquot was carbonated after 5½ hours.

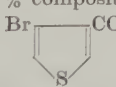
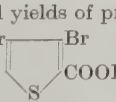
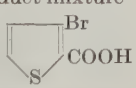
If the reaction mixture is allowed to rise from −70° to room temperature, there is a tendency for the initial product to transform to a mixture consisting of 2-thienyllithium and 3-bromo-2-thienyllithium (Table 2, exp. 8); in other words 3-bromothiophene behaves, in principle, just as the dibromothiophene mentioned above. If the reaction mixture is allowed to stand at room temperature for a long time (24 hours), the acid separates as a difficultly crystallisable oil. Though it was not possible to characterise this product in a satisfactory manner, it might contain, according to some evidence presented by IR-spectroscopy, unsaturated aliphatic products.

When the halide is added to *n*-butyllithium at room temperature and carbonated after a reaction time of about 5 minutes, 3-thiophenecarboxylic acid is mainly obtained, contaminated with smaller amounts of 3-bromo-2-thiophenecarboxylic acid (Table 2, exp. 5, 6 and 7). After standing at room temperature for several hours, the acid mixture separates as an oil which cannot be satisfactorily characterised by IR-spectroscopy.

Reactions with 3,4-dibromothiophene

When the halide is added to *n*-butyllithium at −70°, halogen–metal interconversion occurs and 3-bromo-4-thiophenecarboxylic acid is isolated in good yield after carbonation (Table 3, exp. 1). The α-hydrogens in this halide are more vulnerable than in the above-mentioned bromothiophenes, hence special care has to be taken in the experimental conditions. Local concentration and heating give rise to metalated products, especially if the "reverse" addition is employed. The 3-bromo-4-thienyllithium is fairly stable at −70°, only 8 % having undergone further transformations after $10\frac{1}{2}$ hours (Table 3, exp. 3).

Table 3. Reaction between *n*-C_4H_9Li and  .

No. of exp.	Moles of halide	Final conc.[a] of halide	Time, −70°	Time, room temp.	% composition and yields of product mixture[b]: 		
1	0.04	0.69	5 min		100(80)		
2[c]	0.04	0.48	5 min		98(80)	2(1)	
3	0.05	0.50	10 h 30 min		92(73)	8(5)	
4	0.20	0.68	5 min	16 h	23(16)		77(53)
5	0.16	0.64	5 min	16 h	6(4)	13(7)	81(59)
6	0.10	0.83	30 min	22 h		15(7)	85(50)
7	0.025	0.36		5 min	5(2)	95(34)	
8	0.10	0.75		3 h		100(17)	
9	0.05	0.49		5 h 45 min		100(22)	
10	0.05	0.49		6 h		100(24)	

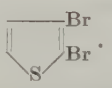
[a] Moles/liter (after mixing the reactants).
[b] Yields in parenthesis.
[c] "Reverse" addition.

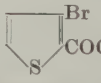
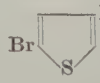
If the temperature of the reaction mixture is allowed to rise from −70° to that of the room, the general trend followed by the other bromothiophenes is observed here as well, namely, 3-bromo-4-thienyllithium tends to transform to a mixture consisting of 3,4-dibromo-2-thienyllithium and 3-bromo-2-thienyllithium (Table 3, exp. 5 and 6). The processes whereby this transformation occurs may be similar to those taking place in the case of the 2,4-isomer.

If the halide is added to the *n*-butyllithium at room temperature, metalation occurs almost exclusively and practically pure 3,4-dibromo-2-thiophenecarboxylic acid can be generally isolated after carbonating the reaction mixture (Table 3, exp. 7–10). The almost complete absence of 3-bromo-4-thiophenecarboxylic acid in the reaction product evidences the enhanced reactivity of the α-hydrogens, whilst the complete absence of rearranged product indicates that the metalation occurs directly through *n*-butyllithium rather than via a 2-stage mechanism, though the evidence is not conclusive. The low yields, together with the strong smell reminiscent of hydrogen sulphide of the reaction mixture, point to extensive decomposition with ring opening of the thiophene nucleus.

Reactions with 2,3-dibromothiophene

An important difference between this compound and the other bromothiophenes mentioned lies in the fact that in the former, the exchangeable α-halogen is situated at the most acidic site, hence 3-bromo-2-thienyllithium will be more stable than

Table 4. Reaction between *n*-C_4H_9Li and  .

No of exp.	Aliquot no.	Moles of halide	Final conc.[a] of halide	Time, −70°	Time, room temp.	% composition and yields of product mixture[b] 	
1	I	0.075	0.79	5 min		100	
	II			2 h 30 min		100	
	III			10 h		100	
2		0.025	0.42	10 h 15 min		100(70)	
3	I	0.075	0.34	5 min		100(65)	
	II				23 h	61(40)	39(19)
4	I	0.05	0.49	5 min		100	
	II				24 h	100	
5		0.20	0.67	5 min	16 h	100(90)	
6		0.025	0.36		5 min	97(66)	3(2)
7	I	0.05	0.42		5 min	100(52)	
	II				4 h 30 min	86(59)	14(7)
8	I	0.05	0.49		5 min	85(50)	15(6)
	II				5 h		100(47)
9	I	0.075	0.50		1 h 45 min	32	68
	II				3 h 45 min	8	92
	III				5 h 30 min	5	95

[a] Moles/liter (after mixing the reactants).
[b] Yields in parenthesis.

the isomeric 4-bromo-2-thienyllithium; and indeed, it was found that no rearrangement occurs in the reactions involving this compound.

When the halide was added to *n*-butyllithium at −70°, halogen-metal interconversion occurred, giving 3-bromo-2-thienyllithium in fairly good yields, which was quite stable at −70° for at least 10 hours (Table 4, exp. 1 and 2).

In three independent experiments, the reaction mixture formed at −70° was allowed to warm up to room temperature. In two of the instances (Table 4, exp. 4 and 5), no change occurred with change in temperature and 3-bromo-2-thiophenecarboxylic acid was isolated in good yield upon carbonation after 24 and 16 hours at room temperature. In one case, however (Table 4, exp. 3), the surprising and completely unexpected observation was made that some of the 3-bromo-2-thienyllithium formed at −70° had disappeared with the simultaneous appearance of 2,4-dibromo-5-thienyllithium. It had been expected that, if a dibromothienyllithium were to be formed at all, it would be the 2,3-dibromo-5-thienyllithium and not the 2,4-isomer. However, as the latter is presumably the thermodynamically most stable dibromothienyllithium, it might well be that this compound would eventually be formed from the less stable 2,3-dibromo-5-thienyllithium by a series of involved reactions.

It is seen (Table 4, exp. 6–9) that pure, or almost pure, 3-bromo-2-thiophenecarboxylic acid can be obtained by carbonating the reaction mixture shortly after mixing the reactants at room temperature, but after longer reaction times this transforms progressively to the metalated product.

Experimental

General procedure for the reaction between n-butyllithium and bromothiophenes

Into a nitrogen-swept 3-necked flask, equipped with stirrer, condenser, dropping funnel and calcium chloride guard tube was introduced ethereal *n*-butyllithium, prepared in the usual way [14] from *n*-butyl chloride and lithium, the normality of which was determined by acid titration. Ethereal bromothiophene was added dropwise during ca. 5 minutes to the *n*-butyllithium, either at −70° or at room temperature, as the case may be. The reaction mixture, or aliquots thereof, was poured onto dry-ice covered with ether, after standing for various times at the temperatures indicated in the tables. The carbonated reaction mixture was treated with water, the aqueous phase separated from the ether phase and the latter extracted with two portions of 2 *N* sodium carbonate solution. The combined aqueous phase was acidified with dilute hydrochloric acid, the precipitate was filtered off, washed repeatedly with small quantities of water till the washings gave only a weak reaction with silver nitrate, and air-dried.

In some of the experiments, the ethereal phase was fractionated and the neutral products examined. For example, fractionation of the organic layer (Table 1, exp. 14) yielded, besides *n*-butyl bromide, 2.3 g of impure 3-bromothiophene, representing a maximum yield of 7 %. A high-boiling residue (1.1 g) remained in the distillation flask; if this consisted entirely of starting material (2,4-dibromothiophene), it represents a maximum yield of 2.3 % recovered halide.

Unless stated otherwise, a 10 % excess of *n*-butyllithium was used in these experiments.

Estimation of the composition of the reaction products

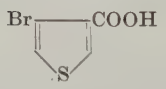
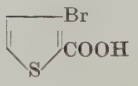
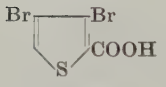
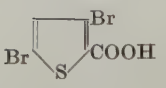
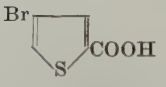
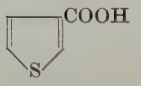
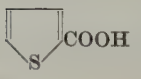
The dried reaction product was analysed by IR-spectroscopy (10 % in acetone) and in some cases, by titration and by NMR-spectroscopy as well.

The isomeric disubstituted thiophenes can be conveniently identified by means of NMR-spectroscopy, since the ring hydrogen spectra of these compounds consist of two doublets having characteristic coupling constants and shifts [15]. The presence of a trisubstituted thiophene can be easily recognised by its single characteristic line. However, IR-spectroscopy is more accurate for quantitative determinations of the composition of mixtures.

In many of the reactions described in the present study, various 2-component mixtures were obtained. To estimate the percentage composition of these mixtures, a series of calibration curves from mixtures of known composition were prepared. In these curves, the ratio of the absorption of a characteristic peak of the one component to that of the other component was plotted as a function of composition. By determining the ratio of two such peaks in the IR-spectrum of the unknown mixture, its composition could be readily estimated.

As seen from the tables, 3-component mixtures were obtained in some of the experiments; since the composition of these mixtures were determined by means of curves drawn from 2-component mixtures, the figures cited in these cases represent a rough approximation only.

Table 5. Absorption peaks used in IR-analyses.

Compound	Characteristic distinguishing peaks (μ)			
	6.67,	8.95,	10.28,	13.75
	8.65,	11.25,	12.87,	13.57
	7.6,	11.1,	14,75	
	10.1,	11.82		
	11.4,	12.05		
	11.4,	12.05,	14.15	
	9.68,	11.55		

The characteristic peaks used in the preparation of the calibration curves are collected in Table 5.

The identity of the two isomeric dibromothiophenecarboxylic acids obtained in this study was established beyond doubt by the elementary analysis of their purified samples and comparison of their physical properties and spectra with authentic samples.

The yields of the dibromothiophenecarboxylic acids were calculated on the assumption that one mole of halide gave rise to one mole of the dibromothiophenecarboxylic acid. This, of course, is only true if the bromo-acid is formed exclusively by direct metalation with *n*-butyllithium; if a 2-stage mechanism *only* is operative, one mole of bromo-acid is derived from two moles of halide, in which case the true yield will be double that quoted in the tables. Thus the yields actually cited represent minimum yields, and will be higher if both routes are operative.

The percentage composition of the product mixture represents percentage by weight.

In some of the experiments, the yields were not determined; however, they are approximately known from other similar experiments carried out under comparable conditions.

The pertinent data concerning the experiments carried out in this study are collected in Tables 1–4.

A Perkin Elmer Infracord spectrophotometer was used for the IR-analyses. The NMR-spectra were obtained at 40 Mc/s with a Varian Associates model V-4300 B high-resolution NMR-spectrometer and a flux-stabilized 12 in. electromagnet from the same company.

SUMMARY

The transformations of 4-bromo-2-thienyllithium, 3-bromo-2-thienyllithium, 3-bromo-4-thienyllithium and of 3-thienyllithium, obtained through halogen-metal interconversion by reacting the appropriate bromothiophene with *n*-butyllithium at $-70°$, have been investigated. These organo-lithium compounds are stable at $-70°$, and hence are very useful intermediates for the preparation of a variety of substituted thiophenes. When the reaction mixture is allowed to rise from $-70°$ to room temperature, there is a general tendency for the initial reaction product to be transformed to a mixture consisting of rearranged and metalated product. Thus 4-bromo-2-thienyllithium transforms to a mixture of 3-bromo-2-thienyllithium and 2,4-dibromo-5-thienyllithium, 3-bromo-4-thienyllithium gives rise to 3-bromo-2-thienyllithium and 3,4-dibromo-2-thienyllithium, 3-thienyllithium changes to 2-thienyllithium and 3-bromo-2-thienyllithium, whilst 3-bromo-2-thienyllithium tends to transform to 2,4-dibromo-5-thienyllithium.

When the reactants are mixed at room temperature, halogen–metal interconversion and metalation seem to occur concurrently to different degrees, and mixtures are obtained. The component obtained through halogen–metal interconversion eventually tends to undergo the transformations described above.

Thus it seems that these thienyllithium derivatives tend to undergo a series of transformations ultimately leading to the derivative, in which the lithium occupies the most "acidic" position.

The mechanism whereby the rearrangement and the metalation might occur is discussed.

ACKNOWLEDGEMENTS

The authors wish to express their thanks to Professor Arne Fredga for his interest in this work and for all facilities put at their disposal. Thanks are also due to Dr R. A. Hoffman for

recording the NMR-spectra. The elementary analyses were carried out at the Analytical Department of the Chemical Institute. A grant from the *Swedish Natural Science Research Council* to S. G. is gratefully acknowledged.

Chemical Institute, University of Uppsala, Uppsala, Sweden, February 1961.

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Tryckt den 21 september 1961

Uppsala 1961. Almqvist & Wiksells Boktryckeri AB

ARKIV FÖR KEMI Band 18 nr 7

Communicated 8 March 1961 by Arne Fredga and Kai Siegbahn

On the coupling constants of pyrroles

By Salo Gronowitz, Anna-Britta Hörnfeldt, Bo Gestblom and Ragnar A. Hoffman

With 7 figures in the text

Introduction

It has been demonstrated that a knowledge of the characteristic coupling constants of thiophenes [1, 2] is exceedingly useful for structure determination of unknown thiophenes [2, 3, 4, 5] and for assignments of the various ring-hydrogen bands to specific hydrogens [1, 6], making possible the study of solvent and substituent effects [7] on the various shifts in the thiophene ring.

Comparing the coupling constants of various monocyclic aromatic systems (cf. Table 5 of ref. 2), one finds some minor but significant differences between thiophenes, furans and pyrroles, which may provide information on the differences in the electronic structure of these related π-excessive [8] heteroaromatic compounds. A detailed theoretical treatment of these different couplings must await more precise determination of the coupling constants in furans and pyrroles, where no value for J_{25} has yet been published and where the magnitudes of the other ring-coupling constants are not very accurately known. For example, the coupling constants in pyrroles were obtained by Abraham and Bernstein [9] for a few compounds in which several of the couplings are nearly equal and could not be accurately determined, as has already been pointed out by these authors [9].

Elimination of couplings to the N-hydrogen of pyrroles

The NMR-study of pyrroles is more complicated than that of thiophenes or furans, due to the presence of the N-hydrogen, which displays couplings to the α- and β-hydrogens of about the same magnitude as the other ring-coupling constants [9]. In the case of pyrrole, Abraham and Bernstein [9] overcome this difficulty through deuteration. We found that these couplings are easily eliminated through base-catalyzed prototropy, thus making the NMR-study of pyrroles accessible with no more difficulties than those encountered in the ordinary three-spin systems of monosubstituted thiophenes and furans.

A pyrrole containing two electron-attracting substituents such as 2,5-dithiocyanopyrrole [10, 11, 12], when dissolved in solvents such as *N*,*N*-dimethyl formamide or dimethyl sulphoxide, undergoes prototropy rapidly enough to eliminate the

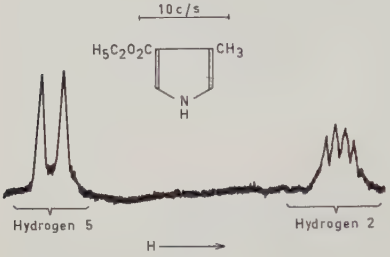

Fig. 1.

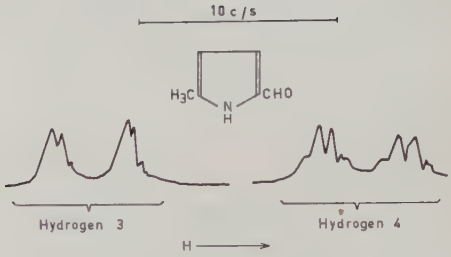

Fig. 2.

Fig. 1. Ring-proton resonance spectrum at 40 Mc/s of 3-methyl-4-carbethoxypyrrole in 22 % dioxane solution containing a few per cent piperidine. The N–H band is not shown.

Fig. 2. Ring-proton resonance spectrum at 40 Mc/s of 5-methyl-2-pyrrolealdehyde in 29 % dioxane solution containing a few per cent piperidine. The N–H band is not shown.

coupling between the N-hydrogen and the other ring-hydrogens, giving a single resonance peak for the two equivalent ring-hydrogens, while in solvents such as acetone and dioxane, a doublet ($J = 2.45$–2.60 c/s) is observed [11]. This difference indicates greater basicities of the two first-mentioned solvents over the two latter (assuming parallelism between rate of protolysis and basicity). The acidity of the N-hydrogen in pyrroles containing only one electron-attracting substituent is so reduced as to require the addition of traces of a base (such as piperidine) for the elimination of the NH-coupling. In order to eliminate the NH-couplings in pyrrole and (even more so) in 2-methylpyrrole larger amounts of piperidine are required and in 2,5-dimethylpyrrole the splittings of the β-hydrogens (due to $J_{13} = J_{14} = 2.7$ c/s [9]) persisted even in dilute piperidine solution.

The increasing difficulties encountered in these decoupling experiments reflect the decreasing acidities of the pyrroles in this series, in agreement with the well-known dependence of the acidity on the electron-attracting or electron-donating properties of the substituents.

NMR-spectra

The decoupled spectrum of pyrrole in 34 % dioxane solution is very similar to that of *N*-deuteropyrrole in 20 % acetone solution [9], the shift between the α- and β-hydrogens having a value of 0.56 p.p.m. The lower field resonance is assigned to the α-hydrogens.

An unambiguous value of the previously unknown coupling J_{25} in pyrroles can be obtained from the spectrum of an unsymmetrically 3,4-disubstituted compound. The ring-proton spectrum of 3-methyl-4-carbethoxypyrrole (Fig. 1) obtained in dioxane solution containing traces of piperidine, consists of a low field doublet ($J_{25} = 2.20$ c/s), assigned to hydrogen 5, and a high field sextet assigned to hydrogen 2. The $-I-M$ effect of the carbethoxy group should unshield the 5-hydrogen more than the 2-hydrogen and similarly the $+I$ effect of the methyl group should preferentially shield the 2-hydrogen (for a detailed discussion of substituent effect on chemical shifts cf. refs. 7 and 13). The 1:3:4:4:3:1 multiplet structure of the 2-hydrogen band arises from the near equality between J_{25} and twice the value of J_{CH_3-2}. The splitting of 1.0 c/s due to J_{CH_3-2} is also observed in the doublet structure of the methyl group band.

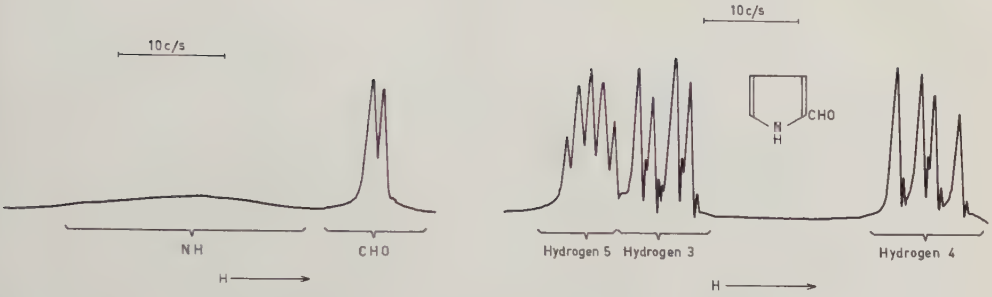

Fig. 3 *a*. Fig. 3 *b*.

Fig. 3. NMR-spectrum at 40 Mc/s of 2-pyrrolealdehyde in 34 % dioxane solution containing traces of piperidine. (*a*) The aldehyde and N-hydrogen bands. (*b*) The bands of hydrogens 3, 4 and 5.

Similarly, a value of J_{45} (3.10 c/s) is obtained from the ring-proton spectrum of 2-methyl-3-carbethoxypyrrole which after NH-decoupling, consists of two closely spaced doublets.

The low-field band in the ring-proton spectrum of 5-methyl-2-pyrrolealdehyde (Fig. 2) is assigned to hydrogen 3 and the high field band to hydrogen 4, the assignments being made on the grounds of chemical shifts. The large doublet splitting (3.75 c/s) is assigned to J_{34} and the quartet structures of the doublet lines are caused by mutual couplings between the methyl group and ring hydrogens ($J_{CH_3-4} = 0.65$ c/s, $J_{CH_3-3} = 0.45$ c/s). The quartet structure in the methyl band could not be completely resolved, and the aldehyde band consists of a single sharp peak, showing the absence of any coupling ($\gtrsim 0.3$ c/s) between the aldehydic proton and the other protons in the molecule.

The resonance of the aldehydic proton of 2-pyrrolealdehyde, on the other hand, consists of a doublet (Fig. 3*a*) ($J = 1.15$ c/s). Since this splitting was absent in 5-methyl-2-pyrrolealdehyde, we assign it to a coupling J_{CHO-5}. The highest field quartet in the ring-hydrogen spectrum (Fig. 3*b*) is assigned to hydrogen 4 because of its chemical shift. The low field quintet is assigned to hydrogen 5, since this structure can only arise for a proton which is coupled to three other protons with two couplings being nearly equal and half as large as the third one, giving a 1 : 2 : 2 : 2 : 1 quintet. The remaining quartet is consequently assigned to hydrogen 3. The greater shielding of hydrogen 3 over that of hydrogen 5 is to be expected because the preferential unshielding of the 3-hydrogen cannot be large enough to reverse the order of the shifts of α- and β-hydrogens in pyrroles (cf. 2-thiophenealdehyde [7]). From the splittings in the bands of hydrogen 3 and 4 we obtain the values of $J_{34} = 3.80$ c/s, $J_{45} = 2.40$ c/s and $J_{35} = 1.40$ c/s.

In the decoupled spectrum of 2-acetylpyrrole (Fig. 4*a*) the lowest field quartet is assigned to hydrogen 5, the intermediate band to hydrogen 3 and the highest field band to hydrogen 4, the assignments being made by comparison of the substituent effect on the shifts of this compound with those of 2-acetylthiophene [7] and 2-pyrrolealdehyde. This assignment gives values of $J_{34} = 3.75$ c/s, $J_{45} = 2.40$ c/s and $J_{35} = 1.35$ c/s, being the same as those observed in 2-pyrrolealdehyde.

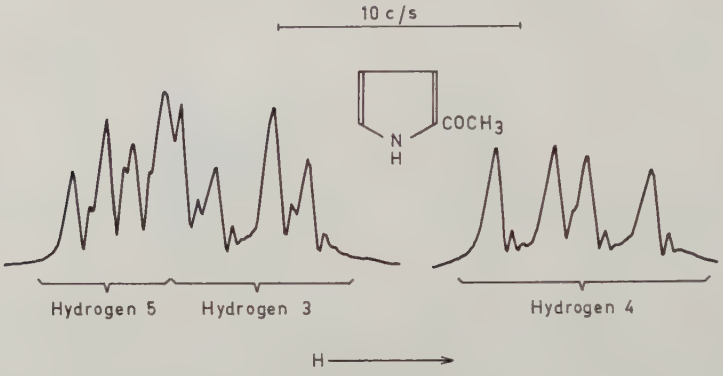

Fig. 4 *a*.

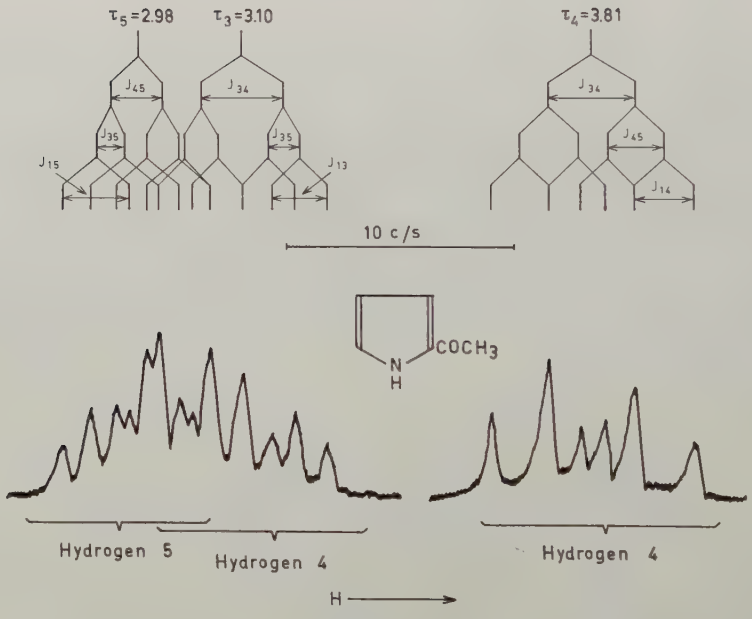

Fig. 4 *b*.

Fig. 4. Ring-proton resonance spectrum at 40 Mc/s of 2-acetylpyrrole. The N–H band is not shown. (*a*) 29 % dioxane solution containing traces of piperidine. (*b*) 25 % dioxane solution.

Having identified the lines in the decoupled spectrum, we are now able to assign all the lines in the complex spectrum of 2-acetylpyrrole (Fig. 4*b*). An unambiguous interpretation of the complex fine structure in the overlapping 3- and 5-hydrogen bands without the preceding interpretation of the decoupled spectrum would have been more difficult. The undecoupled spectrum of 2-pyrrolealdehyde is too complex to be resolved sufficiently to permit a complete assignment of the lines in the 3- and

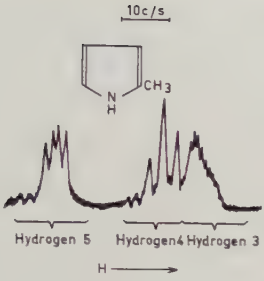

Fig. 5.

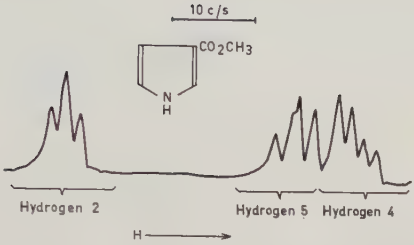

Fig. 6.

Fig. 5. Ring-proton resonance spectrum at 40 Mc/s of 2-methylpyrrole in a solution of dioxane and piperidine. The N–H band is not shown.

Fig. 6. Ring-proton resonance spectrum at 40 Mc/s of 3-carbomethoxypyrrole in 26 % dioxane solution containing traces of piperidine.

5-hydrogen bands, even when the decoupled spectrum has previously been interpreted.

The spectral pattern obtained by us for undecoupled 2-methylpyrrole was identical with that published by Abraham and Bernstein [9]. The decoupled ring-proton spectrum (Fig. 5) consists of a low field quartet assigned to hydrogen 5, a 1:2:1 triplet assigned to hydrogen 4 and a broad band assigned to hydrogen 3. The complex structure of the 3-hydrogen band is caused by unresolved splitting due to J_{CH_3-3}. From the splittings of the 5-hydrogen band we obtain the values of $J_{35} = 1.50 \pm 0.20$ c/s and $J_{45} = 2.45 \pm 0.20$ c/s. The separation of the two outermost components in the hydrogen 4 triplet equals the sum of J_{34} and J_{45} and has the value of 5.85 ± 0.15 c/s. Consequently, we obtain $J_{34} = 3.40 \pm 0.25$ c/s. The lines of hydrogen 4 are apparently somewhat broadened due to J_{CH_3-4}, thereby precluding the resolution of the central triplet line. The values of J_{34} and J_{45} obtained differ considerably from those given by Abraham and Bernstein (2.8 ± 0.1 c/s and 2.6 ± 0.1 c/s respectively [9]). This discrepancy is mainly due to the fact that the splittings observed by these authors constitute averages between these couplings and the NH couplings.

The lowest field band in the ring-proton spectrum of decoupled 3-carbomethoxypyrrole (Fig. 6) can, because of its chemical shift, be assigned to hydrogen 2 with a high degree of certainty. The assignments of the two higher field bands are less obvious, but the most probable assignment is that the resonance of hydrogen 4 occurs at higher field than that of hydrogen 5. From the splittings in the two higher field quartets we obtain the values of $J_{45} = 2.80$ c/s, $J_{25} = 1.95$ c/s and $J_{24} = 1.40$ c/s. The splittings in the triplet have the values of 1.65 c/s representing the average of J_{24} and J_{25}. The values of the coupling constants obtained by applying this assignment are consistent with the values observed in the other compounds.

The present authors have earlier demonstrated the usefulness of methyl substitution for investigating the mechanism of certain long-range couplings [2, 14, 15]. In order to elucidate the mechanism of the NH-couplings, we have studied the NMR-spectra of 2,5-dimethyl-3,4-dithiocyanopyrrole and of 1,2,5-trimethylpyrrole. The methyl groups of the first-mentioned compound give a sharp single peak in dioxane as well as in acetone solution. It is most improbable that the absence of any splitting due to coupling J_{NH-CH_3} is accounted for by rapid prototropy of the N-hydrogen in

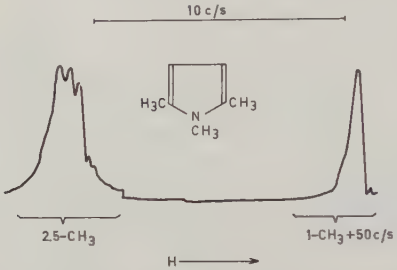

Fig. 7. Methyl group bands in the 40 Mc/s NMR-spectrum of 1,3,5-trimethylpyrrole. The triplet assigned to the α-methyl groups and a (50 c/s) modulation side-band of the N-methyl group are displayed.

these solvents, because the spectra of 2,5-dithiocyanopyrrole (where the N-hydrogen ought to be more acidic) show a well-resolved doublet in these solvents. Thus the π-electron contact mechanism does not contribute significantly to the J_{12}-coupling in pyrroles.

The spectrum of 1,2,5-trimethylpyrrole consists of three bands with relative intensities of 2:3:6, assigned to the ring protons, the N-methyl group and the α-methyl groups respectively. Under higher resolution the α-methyl band shows a triplet with splittings of 0.40 c/s due to coupling with the ring hydrogens, but the N-methyl group remains sharp (Fig. 7).

These results show that the π-electron contact mechanism is of little importance for J_{12} as well as for J_{13}. An N-methyl group should display similar π-electron contact coupling as a directly bound N-hydrogen since hyperfine couplings to the N-methyl group protons and NH protons have been found to be quite similar in the electron-spin resonance spectra of Wurster's blue ions [16, 17, 18] (cf. the arguments presented in ref. 14).

Discussion

The couplings constants in the above-mentioned compounds (cf. Table 1) fall into four distinct ranges: $J_{34} = 3.40$–3.80 c/s, $J_{45} = 2.40$–3.10 c/s, $J_{25} = 1.95$–2.20 c/s and $J_{35} = 1.35$–1.50 c/s. The couplings observed in the spectra of NH decoupled pyrroles can therefore be used for assigning the various bands in monosubstituted pyrroles to specific hydrogens and for structure determination of unknown pyrroles, providing that the observed ranges of the various couplings remain distinct also when a larger number of compounds are studied. In certain condensed pyrroles a value of 3.5–3.6 c/s has been observed for J_{45} [19]; however, the coupling constants in condensed ring systems need not equal those observed in the corresponding monocyclic rings.

It has earlier been suggested [2] that J_{34} has similar magnitudes in thiophenes, furans and pyrroles. The value (3.4–3.8 c/s) for the pyrroles here studied fall entirely within the range (3.4–4.3 c/s) observed for J_{34} in thiophenes [2]. Furthermore, we have confirmed that J_{45} (2.45–3.10 c/s) in pyrrole is considerably smaller than the corresponding couplings (4.9–5.8 c/s) in thiophenes [2]. The "meta"-couplings (J_{24}, J_{35}) have similar magnitudes in pyrroles and thiophenes.

We have earlier pointed out [2] that the smallness of the "meta"-coupling across nitrogen in certain heterocyclic compounds could hardly be due to the perturbing

Table 1. Coupling constants (c/s) in pyrroles. The values listed were obtained from the observed splittings by applying the third-order perturbation corrections [6] assuming like signs for all the couplings. The errors have the value of ± 0.10 c/s except for the NH-couplings, which are certain to within ± 0.15 c/s and for 2-methylpyrrole (cf. text).

Substituents	J_{34}	J_{45}	J_{35}/J_{24}	J_{25}	Other couplings		
3-CH_3, 4-$CO_2C_2H_5$	—	—	—	2.20	$J_{15}=3.05$		$J_{CH_3\text{-}2}=1.00$
2-CH_3, 3-$CO_2C_2H_5$	—	3.10	—	—			
2-CHO, 5-CH_3	3.75	—	—	—		$J_{CH_3\text{-}4}=0.65$;	$J_{CH_3\text{-}3}=0.45$
2-CHO	3.80	2.40	1.40	—	$J_{14}\approx J_{45}$		$J_{CHO\text{-}5}=1.15$
2-$COCH_3$	3.75	2.40	1.35	—	$J_{13}=2.50$;	$J_{14}\approx J_{45}$	$J_{15}=3.00$;
2-CH_3	3.40	2.45	1.50	—			
3-CO_2CH_3	—	2.80	1.40	1.95			

effect of the nitrogen quadrupole relaxation [20] and have suggested that the magnitude of this coupling might depend upon the hybridization of the nitrogen atom. The value of J_{25} (1.95–2.20 c/s) obtained illustrates this dependence. It has similar magnitude as the "meta"-coupling across carbon in monocyclic ring-systems in general [2] and across oxygen in furans.[1]

As in the thiophenes, a 2-methyl group in pyrrole has a coupling to the 3-hydrogen (0.65 c/s) and a smaller coupling to the 4-hydrogen (0.45 c/s). Similarly, the 3-methyl group couples to the 2-hydrogen with 1.0 c/s. A possible coupling $J_{CH_3\text{-}5}$ in 3-methyl-4-carbethoxypyrrole could not be resolved. As in furfural and 2-thiophenealdehyde the aldehydic proton of 2-pyrrolealdehyde couples to the 5-position (1.15 c/s), whilst the absence of mutual couplings between the two side-chains in 5-methyl-2-pyrrolealdehyde is parallelled by the absence of such couplings in 5-alkylsubstituted 2-thiophene- [2] and 2-furanaldehydes [9]. Thus the side-chain couplings in the five-membered heteroaromatics follow the same pattern, and similar mechanisms for the various side-chain couplings should be operative for all these ring systems.

The chemical shifts in dioxane solution are given in Table 2. These shift values do not permit a detailed discussion of substituent effects on the chemical shifts of pyrroles, since solvent interactions are partially responsible for these shifts. However, the unshielding effects of electron-attracting substituents and the shielding effects of electron-releasing substituents become evident by comparing the observed shifts with those of the α- and β-hydrogens of pyrrole respectively. A quantitative comparison of the chemical shifts caused by the substituents in pyrroles with those observed in thiophenes (Table 3) shows great similarities despite the use of different solvents.

Experimental

The NMR-spectra were obtained with a Varian Associates model V-4300 B high-resolution spectrometer, operating at 40.00 Mc/s and a flux-stabilized 12 in. magnet equipped with shim-coils from the same company. The magnet sweep was calibrated with the modulation side-band technique using a 50.00 ± 0.05 c/s modulation fre-

[1] J_{25} in 3-iodofuran has the value of 1.55 c/s [21] and Abraham and Bernstein [22] quote an average of 1.55 c/s for this coupling.

Table 2. Chemical shifts (ppm) of pyrroles in dioxane solution. The shifts are converted to τ-values [24]. The resonance of the N-hydrogen occurs as a broad band at very low field (usually $< +1.0$ ppm).

Substituents	Concentration %	τ_2	τ_3	τ_4	τ_5	Side chains
None	34	3.27	3.83	3.83	3.27	
3-CH_3, 4-$CO_2C_2H_5$	22	3.53	—	—	2.70	$\tau_{3\text{-}CH_3} = 7.76$
2-CH_3, 3-$CO_2C_2H_5$	27	—	—	3.56[a]	3.50[a]	
2-CHO, 5-CH_3	29	—	3.07	4.01	—	$\tau_{CHO} = 0.70$; $\tau_{CH_3} = 7.72$
2-CHO	34	—	3.07	3.74	2.87	$\tau_{CHO} = 0.53$
2-$COCH_3$	29	—	3.10	3.81	2.98	
2-CH_3	32	—	4.25	4.06	3.48	$\tau_{CH_3} = 7.76$
3-CO_2CH_3	26	2.56	—	3.43	3.24	
2,5-$(CH_3)_2$; 3,4-$(SCN)_2$	10	—	—	—	—	$\tau_{CH_3} = 7.70$
1-CH_3, 2,5-$(CH_3)_2$	39[b]	—	4.41	4.41	—	$\tau_{2,5\text{-}CH_3} = 7.99$; $\tau_{1\text{-}CH_3} = 6.95$

[a] The assignment is uncertain.
[b] These shifts were obtained in cyclohexane solution.

quency obtained from the power-line. For the large shifts several side-bands, produced by increasing the modulation field [23] were used and the shifts were then measured on the recorder chart by interpolation.

The chemical shifts were obtained as f_i-values [6] relative to the solvent and converted to τ-values [24]. The τ-value of dioxane (6.430 ± 0.005 ppm) was obtained from a spectrum of dioxane containing 2 % of tetramethylsilane.

Pyrrole, b.p. 131°, $n_D^{20} = 1.5002$, was a commercial product.

2-Pyrrolealdehyde, m.p. 43–45°, was prepared through the Vilsmeier formylation of pyrrole [25].

2-Methylpyrrole, b.p. 146–147°, $n_D^{24} = 1.5024$, was prepared through the Wolf–Kishner reduction of 2-pyrrolealdehyde [26].

5-Methyl-2-pyrrolealdehyde. This compound was prepared in a new way analogously to 2-pyrrolealdehyde. From 3.5 g (0.043 moles) of 2-methylpyrrole in 12 ml of ethylene chloride, 3.8 g of *N*,*N*-dimethylformamide and 8.0 g of phosphorous oxychloride was obtained 3.7 g (79 %) of 5-methyl-2-pyrrolealdehyde, m.p. 65–70°. After two recrystallisations from petrol–ether, 2.7 g (57 %) of pure aldehyde, m.p. 71–72°, was obtained. (Literature value [27] m.p. 70°.)

2,5-Dimethylpyrrole, b.p. 76–80°/24 mm Hg, $n_D^{20} = 1.5028$, was prepared through the reaction of acetonylacetone with ammonium carbonate [28].

1,2,5-Trimethylpyrrole, b.p. 54–56°/9 mm Hg, $n_D^{20} = 1.4994$, was prepared through the reaction of acetonylacetone with methylamine [29].

2-Methyl-3-carbethoxypyrrole, m.p. 79–80°, was prepared through the reaction of ethyl acetoacetate and ethyl α,β-dichloroethyl ether in aqueous ammonia [30].

3-Carbomethoxypyrrole, m.p. 87.5–88.5°, was prepared through the condensation of aminoacetaldehyde with methyl formylacetate [31].

2-Acetylpyrrole, m.p. 90–91°, was prepared through the reaction of pyrrolemagnesium iodide with acetyl chloride [32].

2,5-Dimethyl-3,4-dithiocyanopyrrole, m.p. 138.5–139.5°, was prepared through the thiocyanation of 2,5-dimethylpyrrole [11].

Table 3. Chemical shifts (ppm) of the ring hydrogens in some mono-substituted pyrroles relative to the α- and β-hydrogens in pyrrole and the shifts in the corresponding thiophenes [7] relative to the α- and β-hydrogens in thiophene. The values for the pyrroles were obtained from Table 2.

Compound	δ_2	δ_3	δ_4	δ_5
2-methylpyrrole	—	+0.42	+0.23	+0.21
2-methylthiophene	—	+0.37	+0.24	+0.28
2-pyrrolealdehyde	—	−0.76	−0.09	−0.40
2-thiophenealdehyde	—	−0.65	−0.10	−0.45
2-acetylpyrrole	—	−0.73	−0.02	−0.29
2-acetylthiophene	—	−0.57	0.00	−0.28
3-carbomethoxypyrrole	−0.71	—	−0.40	−0.03
3-carbomethoxythiophene	−0.78	—	−0.47	+0.05

3-Methyl-4-carbethoxypyrrole, m.p. 76–78°, was prepared through a five-step synthesis. 2,4-dicarbethoxy-3-methyl-5-carboxypyrrole was prepared from ethyl acetoacetate and oxalylacetic acid by the Knorr synthesis [33]. Alkaline hydrolysis gave 2,5-dicarboxy-3-methyl-4-carbethoxypyrrole [34], which was iododecarboxylated to 2,5-diiodo-3-methyl-4-carbethoxypyrrole [34]. Hydrogenation over Raney-nickel gave the desired 3,4-unsymmetrically substituted pyrrole [33].

SUMMARY

The ring-coupling constants in simple monosubstituted and disubstituted pyrroles are reported. It has been observed that addition of small amounts of piperidine eliminates the couplings between the N-hydrogen and the other ring hydrogens, reducing the spectra of monosubstituted pyrroles to those of three-spin systems. The ring-coupling constants have the values of J_{34} = 3.40–3.80 c/s, J_{45} = 2.40–3.10 c/s, J_{25} = 1.95–2.20 c/s and J_{24} = 1.35–1.50 c/s. The couplings J_{13} and J_{14} are of similar magnitude as J_{45} and the couplings J_{12} and J_{15} are greater ($\approx$ 3.0 c/s). A discussion on the mechanism of these couplings is given. Couplings between the side-chain protons and ring protons are observed in methylpyrroles and 2-pyrrolealdehyde; $J_{CH_3\text{-}3}$ = 0.65 c/s and $J_{CH_3\text{-}4}$ = 0.45 c/s in 2-methyl-5-pyrrolealdehyde, $J_{CH_3\text{-}2}$ = 1.0 c/s in 3-methyl-4-carbethoxypyrrole and $J_{CHO\text{-}5}$ = 1.15 c/s in 2-pyrrolealdehyde.

ACKNOWLEDGEMENTS

The authors wish to express their thanks to Prof. Arne Fredga and Prof. Kai Siegbahn for their interest in this work and for all facilities put at their disposal. Grants from the *Swedish Natural Science Research Council* to S. G. and from the *Swedish Technical Research Council* to R. A. H. are gratefully acknowledged.

Institute of Chemistry and Institute of Physics, University of Uppsala, February 1961.

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Tryckt den 21 september 1961

Uppsala 1961. Almqvist & Wiksells Boktryckeri AB

ARKIV FÖR KEMI Band 18 nr 8

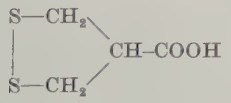
Communicated 12 April 1961 by Arne Fredga

Synthesis of 1-thia-2-selena-cyclopentane-4-carboxylic acid

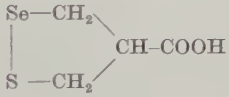
By Göran Bergson and Aija Biezais

The aim of the present paper is to describe a synthesis of 1-thia-2-selena-cyclopentane-4-carboxylic acid (II), a compound which contains a hitherto unknown ring system. The investigation of this acid constitutes one part of a series of studies on the Se–S bond in aliphatic molecules, as compared with the corresponding disulphides (R–S–S–R) and diselenides (R–Se–Se–R). Some experiments with non-cyclic thiolselenenates (R–Se–S–R) have recently been concluded [1] and particular attention has been devoted to their spectral properties.

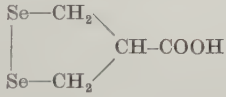
The syntheses of 1,2-dithiolane-4-carboxylic acid (I) and 1,2-diselenolane-4-carboxylic acid (III) have previously been carried out [2, 3]. These compounds are more readily obtained than II since their syntheses can be effected by known methods



I



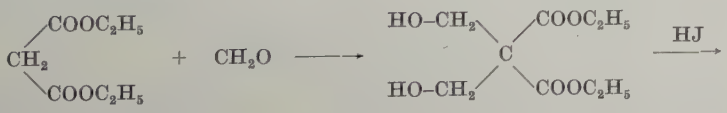
II

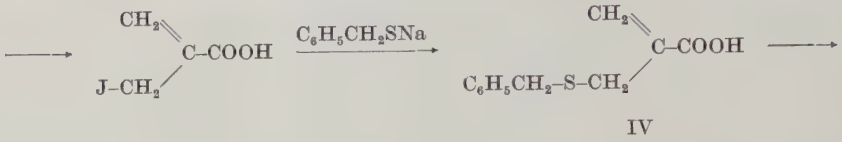


III

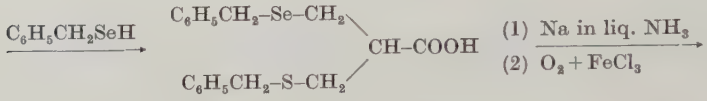
based on the conversion of β,β'-dihalogen-iso-butyric acids to the disulphide or diselenide respectively. Starting with β,β'-diiodo-iso-butyric acid Schotte and Ström [2] converted this compound to β,β'-dithiol-iso-butyric acid via the thiolacetate. The dithiol was then oxidized by air and $FeCl_3$ or by iodine to give a low yield of 1,2-dithiolane-4-carboxylic acid. 1,2-diselenolane-4-carboxylic acid was synthesized from β,β'-dibromo-iso-butyric acid [3] by two alternative routes. The first depended on direct reaction between the bromo-compound and sodiumdiselenide, the second on reaction between the bromo-compound and sodium benzylselenolate, subsequently followed by debenzylation with sodium in liquid ammonia and oxidation of the selenol thereby formed.

Our method for the synthesis of 1-thia-2-selena-cyclopentane-4-carboxylic acid is shown in the following scheme:

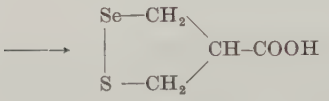




IV



V



II

The condensation between malonic ester and formaldehyde was carried out according to the instructions given by Gault and Roesch [4]. The preparation of α-iodomethyl-acrylic acid from ethyl bis-(hydroxymethyl)-malonate has been described by Welch [5]. Reaction between the sodium salt of α-iodomethyl-acrylic acid and sodium benzylthiolate in dilute ethanolic solution gave S-benzyl-α-(thiolmethyl)-acrylic acid (IV) in 72 % yield. The melting point (92–94°) of this compound was observed to be the same as that found by Corse and Jansen [6] who obtained it from β,β′-dibromo-iso-butyric acid and sodium benzylthiolate. The infrared spectrum of (IV) is given in Fig. 1. It shows the peaks typical of a carboxylic group, an ethylenic double bond and a benzene ring.

The addition of benzylselenol to the ethylenic double bond in (IV) was made in 85 % yield by refluxing the reactants in ether solution under nitrogen, using a small

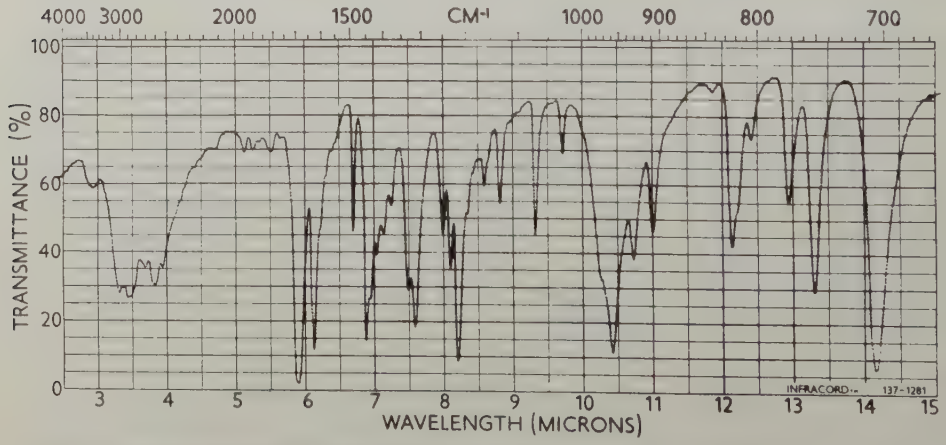


Fig. 1. Infrared spectrum of S-benzyl-α-(methylthiol)-acrylic acid (IV). Solid in KBr.

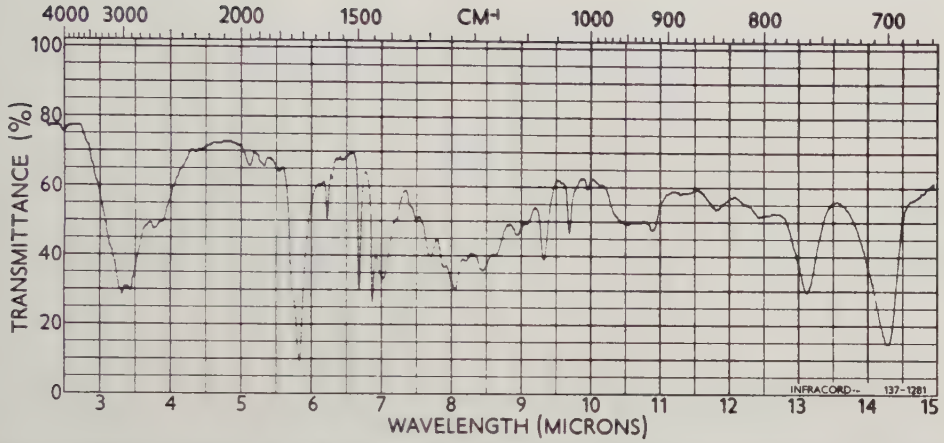


Fig. 2. Infrared spectrum of 1-thiobenzyl-3-selenobenzyl-propane-2-carboxylic acid (V). Liquid between NaCl-plates.

amount of piperidine as a catalyst. The 1-thiobenzyl-3-selenobenzyl-propane-2-carboxylic acid (V) was then recrystallized from toluene and melted at 64.5–66.5°. Corse and Jansen [6] describe dibenzyl-mercapto-iso-butyric acid as a thick oil and dibenzyl-selenol-iso-butyric acid has the same characteristics [3]. We have, however, observed that dibenzyl-mercapto-iso-butyric acid crystallizes after several months in the cold.

The infrared spectrum of (V) is given in Fig. 2. It shows peaks for a carboxylic group and for benzene rings but those for the ethylenic double bond are absent.

The debenzylation of (V) was carried out by means of sodium in liquid ammonia at about $-70°$. The thiol-selenol formed in this way was oxidized by air in very dilute aqueous solution at pH 7–8, using $FeCl_3$ as a catalyst. Formation of polymeric

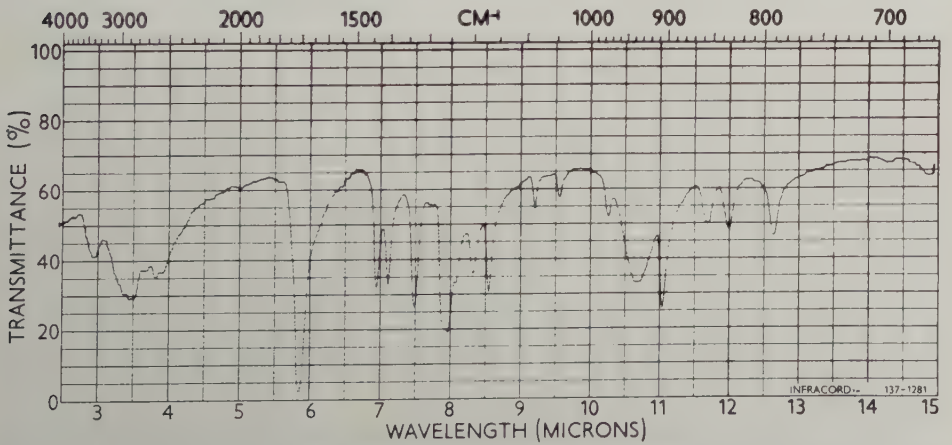


Fig. 3. Infrared spectrum of 1-thia-2-selena-cyclopentane-4-carboxylic acid (II). Solid in KBr.

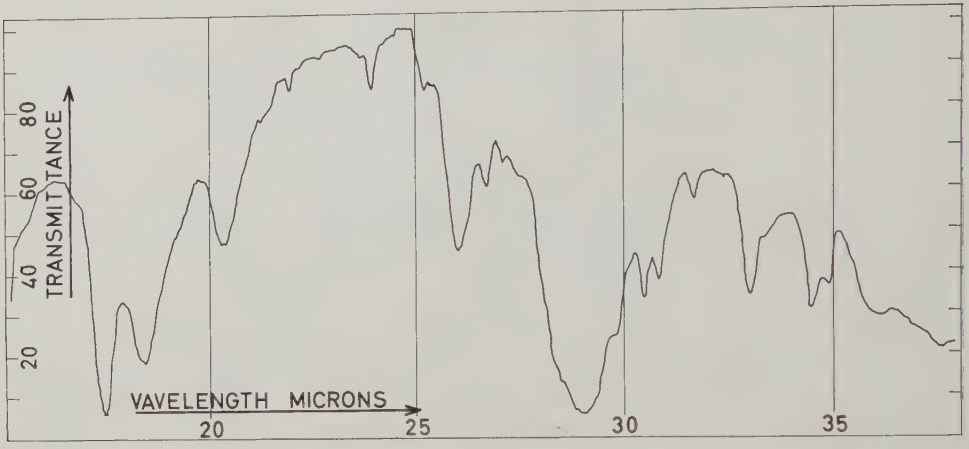
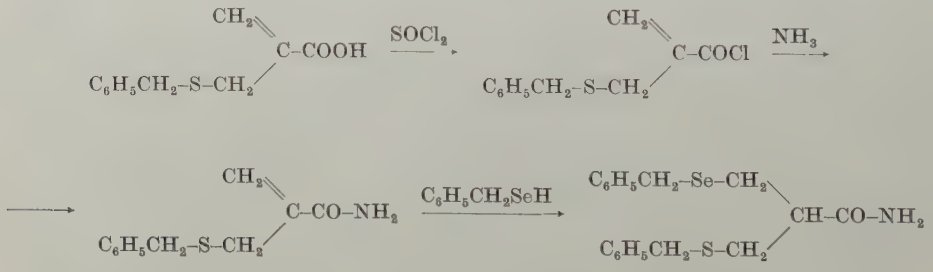


Fig. 4. Infrared spectrum (15–38 mμ) of 1-thia-2-selena-cyclopentane-4-carboxylic acid (II) in Nujol suspension.

material was observed in all our preparations but in the most successful run the five-membered ring (II) was formed in 63 % yield. The 1-thia-2-selena-cyclopentane-4-carboxylic acid formed reddish-yellow crystals (from chloroform-cyclohexane) melting at 105.5–107.5°. The infrared spectrum of (II) is given in Figs. 3 and 4. The peaks characteristic for benzene rings are absent. The long-wave-length spectrum (Fig. 4) is very difficult to interpret. The peak at 17.4 or 18.4 μ may possibly be described in terms of C–Se stretching [7], and that at 29 μ in terms of Se–S stretching. Attempts were also made to modify the synthesis by reacting iodomethyl-acrylic acid with sodium benzylselenolate. The aim was to add benzylthiol to the Se-benzyl-α-(selenolmethyl)-acrylic acid formed in this way. However, this acid proved to be difficult to obtain in a pure form and it was only slightly soluble in ether. This method was therefore abandoned in favour of the original path.

In addition to the above the following sequence of reactions was carried out with the aim of obtaining further derivatives of 1-thia-2-selena-cyclopentane. These experiments will be described later.



The ultraviolet spectrum of (II) shows a characteristic peak at 386 mμ (Fig. 5). The spectra of I and II have exactly the same shape as that of II, but with maxima

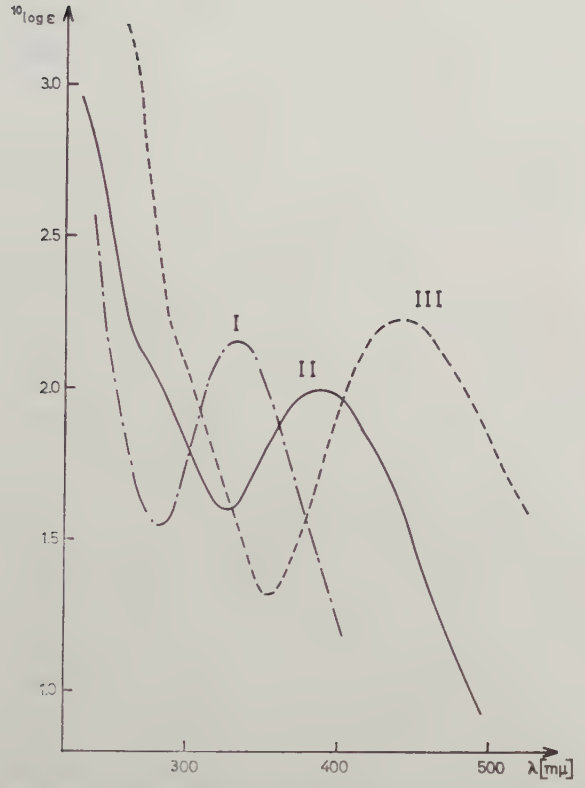


Fig. 5. Ultraviolet spectra of 1,2-dithiolane-4-carboxylic acid [13] (I), 1-thia-2-selena-cyclopentane-4-carboxylic acid (II) and 1,2-diselenolane-4-carboxylic acid (III). (I) and (II) are in ethanolic solution, (III) in chloroform.

at 330 mμ and 440 mμ respectively. The theoretical significance of the observed similarity in ultraviolet spectra of disulphides, thiolselenates and diselenides has been discussed previously [1, 8] and need not be repeated here.

As reported above, polymeric products were formed in the oxidation of propane-1-thiol-3-selenol-2-carboxylic acid. Polymerization of 1-thia-2-selena-cyclopentane-4-carboxylic acid at temperatures just above its melting point has also been observed. Molecular weight determinations with the Rast camphor method showed that at 160–170°C the substance must contain molecules other than the monomer. However, ebullioscopic determination of the molecular weight of the crystalline product (II), dissolved in acetone, showed it to be monomeric. Visual inspection of the melting process in (II) indicates that it first melts at 105.5–107.5° then rapidly polymerizes to a semi-solid mass. At higher temperatures a second, more gradual, melting takes place, an effect indicative of depolymerization. This behaviour is similar to that of cyclic disulphides and diselenides [9]. The structure of the polymer formed from 1-thia-2-selena-cyclopentane and its derivatives is an open question. They may contain units with Se–S bonds only as in (VI) or with S–S and Se–Se bonds as in (VII) or perhaps a mixture of the two alternatives.

$$-CH_2-CH_2-CH_2-Se-S-CH_2-CH_2-CH_2-Se-S-CH_2-CH_2-CH_2-Se-S- \quad \text{(VI)}$$

$$-CH_2-CH_2-CH_2-Se-Se-CH_2-CH_2-CH_2-S-S-CH_2-CH_2-CH_2-Se-Se- \quad \text{(VII)}$$

An investigation of the polymeric 1-thia-2-selena-cyclopentane is planned.

Experimental

Ethyl bis-(hydroxymethyl)-malonate was prepared according to Gault and Roesch [4]. Yield 71 %, m.p. 41–45°.

α-Iodomethyl-acrylic acid was prepared according to Welch [5]. Yield 84 %, m.p. 104–106°.

Benzylthiol was made via a Bunte salt according to Rodionow and Ssuworow [10]. Yield 76 %, b.p. 49–52°/0.8 mm.

Benzylselenol was prepared over benzylmagnesiumchloride according to Painter [11]. Yield 51 %, b.p. 90–92°/14 mm.

S-benzyl-α-(thiolmethyl)-acrylic acid (*IV*). An alcoholic solution of sodium benzylthiolate was prepared from 28.4 g (0.23 mole) of benzylthiol and 5.27 g (0.23 mole) sodium in 90 ml absolute ethanol. This solution was added dropwise to a solution of the sodium salt of α-iodomethyl-acrylic acid prepared from 48.5 g (0.23 mole) α-iodomethyl-acrylic acid and 53.5 g $Na_2CO_3 \cdot 7H_2O$ in 400 ml water. The reaction mixture was allowed to stand at room temperature for 16 h and then acidified with 2-molar HCl. The product separated as slightly yellow crystals, which were filtered and washed with water and petroleum ether and dried in vacuum over sulphuric acid. Yield 34.2 g (72 %), m.p. 86–90°. After recrystallization from petroleum ether the acid was obtained in colourless needle-shaped crystals, m.p. 92–94°. Equivalent weight: Calc: 208.3. Found: 202.6.

1-Thiobenzyl-3-selenobenzyl-propane-2-carboxylic acid (*V*). 46.1 g (0.22 mole) S-benzyl-α-(thiolmethyl)-acrylic acid (IV), 37.9 g (0.22 mole) benzylselenol and 0.5 ml piperidine were dissolved in 250 ml ether. The mixture was refluxed under nitrogen for 3 h, the ether distilled off and the remaining mixture held at 85° for 4 h.

The product crystallized after 4 days. M.p. 57–64°. Yield 71 g (85 %). Recrystallization from toluene gave (V) in the form of colourless crystals, m.p. 64.5–66.5°. Selenium: Calc: 20.8 %. Found: 20.7 %.

1-Thia-2-selena-cyclopentane-4-carboxylic acid (*II*). A solution of 10.0 g (0.026 mole) of (V) in 50 ml toluene was added to about 300 ml of liquid ammonia at about −70°. About 2.5 g of sodium was added in small amounts with mechanical stirring. When the blue sodium colour had persisted for more than 5 minutes, the excess of sodium was destroyed with ammonium chloride. The ammonia was allowed to evaporate and 200 ml water was added to the residue. The aqueous phase was separated, its pH adjusted to 7–8 with hydrochloric acid, and it was then diluted to 800 ml. A trace of $FeCl_3$ was added and the characteristic dark violet colour indicating the presence of thiol groups appeared. Air was passed through the solution for about 12 h and the resulting yellow solution, filtered from 0.15 g of precipitated selenium, was acidified with hydrochloric acid and extracted with 15 × 75 ml of chloroform. The combined extracts were dried with Na_2SO_4 and the solvent removed in vacuum. The residue consisted of reddish-yellow crystals, m.p. 95–105°. Yield 3.2 g (63 %).

Recrystallization from chloroform–cyclohexane gave II with m.p. 105.5–107.5°. Selenium: Calc: 40.1. Found: 39.8.

Equivalent weight: Calc: 197.1. Found: 197.2.
Molecular weight: Calc.: 197.1. Found ebullioscopically in acetone: 168. Cryoscopically in camphor at 164.5°: 374 at 170°: 282.

The selenium analyses were carried out according to Fredga [12]. The infrared spectra in Figs. 1–3 were recorded with a Perkin-Elmer Model 137 spectrometer and that in Fig. 4 with a Perkin-Elmer Model 21 spectrometer equipped with a CsBr prism. The ultraviolet spectrum was taken with a Beckman model DU (220–400 mμ) and model B (400–500 mμ) spectrometer.

SUMMARY

1-Thia-2-selena-cyclopentane-4-carboxylic acid (II) has been synthesized and its spectral properties have been investigated.

ACKNOWLEDGEMENTS

The authors are indebted to Professor Arne Fredga for valuable discussions and for all the facilities placed at their disposal. A grant from the *Swedish Natural Science Research Council* is gratefully acknowledged.

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Tryckt den 21 september 1961

Uppsala 1961. Almqvist & Wiksells Boktryckeri AB

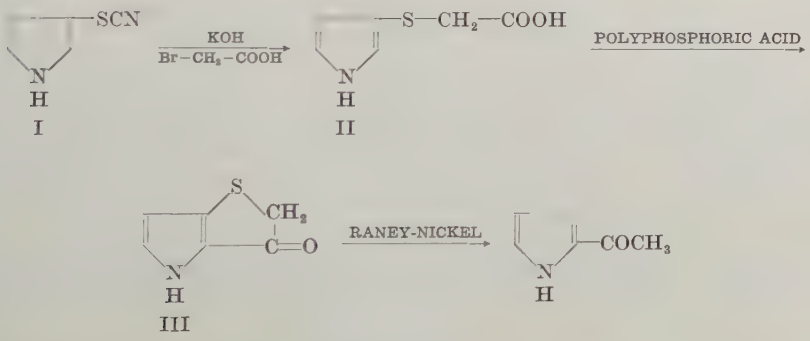
ARKIV FÖR KEMI Band 18 nr 9

Communicated 12 April 1961 by Arne Fredga and Kai Siegbahn

On the structure of the thiocyanation products of pyrrole

By Salo Gronowitz, Anna-Britta Hörnfeldt, Bo Gestblom and Ragnar A. Hoffman

Some years ago, Matteson and Snyder [1, 2] investigated the monothiocyanation of pyrrole in connection with work on the synthesis of thieno[3,2-b]pyrrole. They found that thiocyanogen in methanol at $-70°$, or cupric thiocyanate at 0°, gave a monothiocyanopyrrole. They showed it to be 3-thiocyanopyrrole (I), by converting it to (3-pyrrolylthio)acetic acid (II), which was ring-closed to 2H,3H,-thieno[3,2-b] pyrrole-3-one (III) and desulphurized to 2-acetylpyrrole.



This is a rather unexpected result, since substitution in the β-position of 5-membered heteroaromatics by electrophilic reagents is very exceptional, although it has been shown for imidazole that different electrophilic reagents attack at different positions [3].

In connection with our work on the NMR-spectra of heteroaromatic compounds [4, 5, 6, 7] we have studied the NMR-spectrum of the above compound. As this, however, is anomalous in many respects, casting doubt on the reliability of the chemical evidence for its structure, we will discuss it in some detail.

The NMR-spectrum of thiocyanopyrrole in dioxane solution consists of three bands (A, B and C) with complex fine-structure (Fig. 1). On addition of traces of piperidine, the splittings due to the NH-couplings are eliminated [7] and the spectrum simplifies to that of an ordinary three-spin system consisting of three bands of quartets (Fig. 2). The chemical shifts of the various bands and the ring coupling constants in different solvents are given in Table 1.

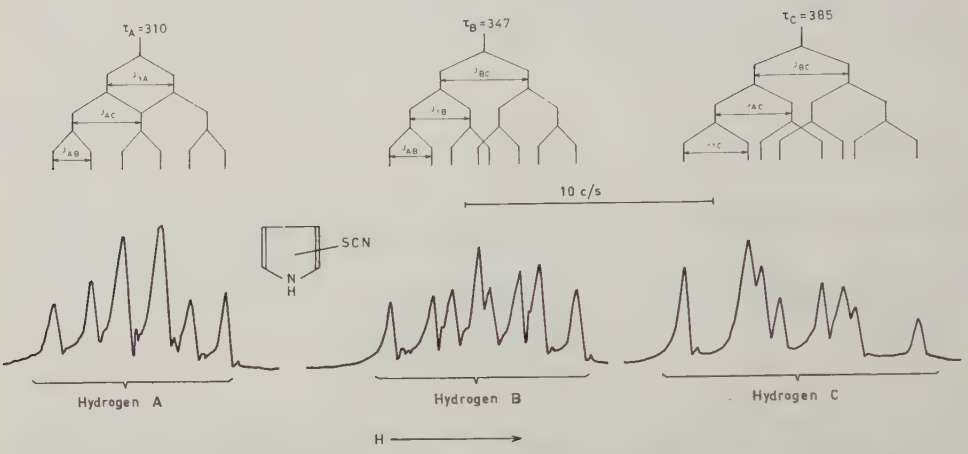


Fig. 1. NMR-spectrum at 40 Mc/s of the monothiocyanation product of pyrrole in 40 % dioxane solution. The broad N–H band is not shown.

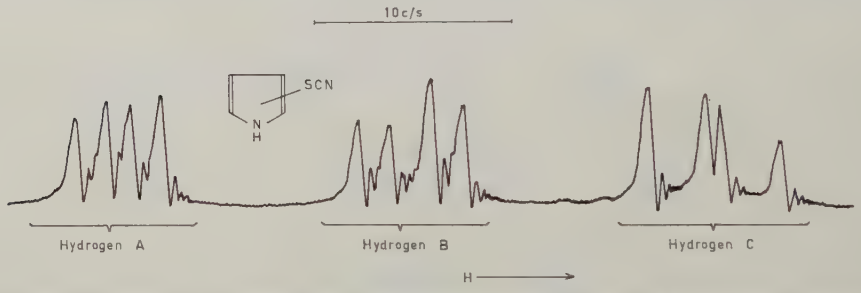


Fig. 2. NMR-spectrum at 40 Mc/s of the monothiocyanation product of pyrrole in 40 % dioxane solution containing traces of piperidine.

Table 1. Chemical shifts (ppm) and coupling constants (c/s) of monothiocyanopyrrole in various solvents.

The chemical shifts were obtained as f_i-values [4] relative to the solvents and converted to τ-values [28]. The shifts of the solvents are: Dioxane, $\tau = 6.430$ ppm; Dimethyl sulfoxide, $\tau = 7.47$ ppm; Cyclohexane, $\tau = 8.558$ ppm. The coupling constants were obtained from the observed splittings by applying the third-order perturbation corrections [4], assuming like sign of all couplings. The uncertainties in the values obtained are ± 0.10 c/s.

Solvent	Concentration weight %	τ_A	τ_B	τ_C	J_{AB}	J_{AC}	J_{BC}	J_{1A}	J_{1B}	J_{1C}
Dimethyl sulfoxide	17	2.94	3.43	3.81	1.55	2.85	3.50	2.80	2.40	2.35
Cyclohexane	76	3.24	3.50	3.87	—	—	—	—	—	—
Dioxane	40	3.10	3.47	3.85	1.50	2.90	3.60	2.85	2.45	2.40

From the spectrum of 3-thiocyanothiophene [5] it is known that a 3-thiocyano group unshields the 2-hydrogen, but does not appreciably affect the shifts of the 4- and 5-hydrogens. We assume that the thiocyano group will have similar effects on the shifts of the α- and β-hydrogens in 3-thiocyanopyrrole, since we have earlier demonstrated [7] the general correspondence of the chemical shifts in monosubstituted pyrroles with those of their thiophene analogues. Therefore we assign the lowest field band (A) to hydrogen 2, the central band (B) to hydrogen 5 and the highest field band to hydrogen 4, being the same order of shifts as in 3-carbomethoxypyrrole [7]. The chemical shifts of the various ring hydrogens as compared to unsubstituted pyrrole are not unreasonable. However, this assignment leads to unacceptable values for J_{25} (1.5 c/s) and J_{45} (3.6 c/s) and especially for J_{24} (2.9 c/s), as the values of J_{25}, J_{45} and J_{24} have been found to fall in the ranges 1.95–2.20 c/s, 2.40–3.10 c/s and 1.35–1.50 c/s [7]. A more reasonable value for J_{24} (1.5 c/s) is obtained by assigning band B to hydrogen 4 and band C to hydrogen 5, but the values obtained for J_{25} (2.9 c/s) and J_{45} (3.6 c/s) are still much larger than any value obtained for these couplings in other pyrroles. Furthermore, this assignment requires that the 3-thiocyano group increases the shielding of hydrogen 5 by 0.6 ppm which seems quite unacceptable (cf. δ_5 -0.05 ppm in 3-thiocyanothiophene [5]). If we discard the natural assumption that the resonance of hydrogen 2 has to occur at lowest field, we may assign band A to hydrogen 5 or hydrogen 4, but this leads to the unacceptable value of 3.6 c/s for J_{24} or J_{25}.

On the other hand, the spectrum observed is in good agreement with that expected for 2-thiocyanopyrrole. In this compound, one can safely predict that the resonance of hydrogen 5 will occur at lower field than that of hydrogen 3, which in turn would be less shielded than hydrogen 4 since the thiocyano group should unshield hydrogen 3 and 5 (cf. 2-thiocyanothiophene [5]). By this assignment we obtain the values of $\delta_3 = -0.36$ ppm, $\delta_4 = -0.02$ ppm and $\delta_5 = -0.17$ ppm for the shifts relative to the α- and β-hydrogens of pyrrole [7] which are similar to those of the shifts of the corresponding hydrogens of 2-thiocyanothiophene relative to thiophene ($\delta_3 = -0.30$ ppm, $\delta_4 = -0.05$ ppm, $\delta_5 = -0.28$ ppm [5]). The shifts are in the same sense as those of 2-pyrrolealdehyde and 2-acetylpyrrole [7], although these substituents are more unshielding in the 3- and 5-positions due to their stronger electron-attracting properties, as is also the case with the thiophene analogues [5]. The values of the coupling constants obtained with this assignment ($J_{34} = 3.6$ c/s, $J_{45} = 2.9$ c/s and $J_{35} = 1.5$ c/s) fall entirely within the ranges of values obtained for these coupling constants in other pyrroles [7].

From the foregoing discussion, it is evident that the NMR-spectrum of the monothiocyanation product of pyrrole is that expected for a 2-substituted compound and hardly compatible with that expected for a 3-substituted pyrrole.

Accepting the chemical proof of the structure of the thiocyanation product of pyrrole, one infers that the generally accepted rules of the constancy of the spin couplings in monocyclic ring systems [6, 8] and the regular dependence of the chemical shifts on substituent effects [5, 8] are not reliable. We hesitate to accept this conclusion and have therefore investigated the structure of the thiocyanation product in other ways.

Additional information on the orientation in thiocyanation can be obtained through the study of the thiocyanation of 2-methylpyrrole. Any one of the three possible monothiocyano-2-methylpyrroles could be identified by means of NMR-spectroscopy, not only because of its characteristic shifts and ring coupling constants

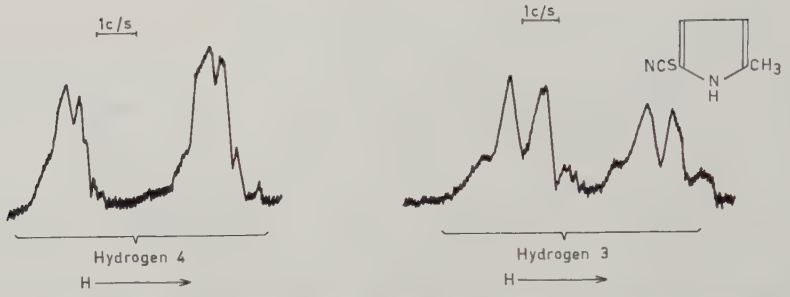


Fig. 3. Ring proton resonance spectrum at 40 Mc/s of 2-methyl-5-thiocyanopyrrole in 27 % dioxane solution, containing small amounts of piperidine. (*a*) The band assigned to hydrogen 4; (*b*) the band assigned to hydrogen 3.

but also because of its methyl-to-ring coupling constants. Upon thiocyanation of 2-methylpyrrole with cupric thiocyanate at 0° we obtained a compound of m.p. 65.5–66°, which proved to be a monothiocyano-2-methylpyrrole by elementary and IR-spectral analyses. The ring proton spectrum of this compound, dissolved in dioxane containing small amounts of piperidine (Figs. 3*a* and 3*b*), consists of two doublets of quartets. The chemical shifts have the values of $\tau = 4.13$ ppm and $\tau = 3.56$ ppm. The doublet splitting has the value of 3.55 ± 0.10 c/s. The larger quartet coupling has the value of 0.80 ± 0.10 c/s and the smaller one a value of 0.35 ± 0.10 c/s.

From this spectrum we conclude that the compound formed is 5-thiocyano-2-methylpyrrole. A large internal shift (0.57 ppm) between the 3- and 4-hydrogens is to be expected, as the thiocyano group should unshield the 4-hydrogen while the methyl group should shield the 3-hydrogen. The resonance of hydrogen 3 occurs at 0.30 ppm towards higher and that of the 4-hydrogen at 0.27 ppm towards lower field than that of the β-hydrogens of pyrrole. The couplings of the methyl group to the 3- and 4-hydrogens have similar values as the corresponding couplings in 2-methyl-5-pyrrolealdehyde [7], the spectrum of which closely resembles that of the present compound, although the resonance of the 4-hydrogen occurs at lower field (0.66 ppm relative pyrrole), due to the stronger unshielding effect of the aldehyde group (cf. Table 2 in ref. [7]). The doublet splitting of 3.55 c/s falls in the range of values observed for J_{34} in pyrroles [7].

The possibility that the compound formed is the 2,4-isomer is excluded by the largeness of the observed splitting, since J_{35} is known to fall in the 1.4–1.5 c/s range [7]. The 2,3-isomer is excluded because of the large internal shift (cf. the value of 0.06 ppm for the shift between the 4- and 5-hydrogen in 2-methyl-3-carbethoxypyrrole [7]) and because of the presence of two methyl-to-ring couplings, the larger of which can only be due to $J_{CH_3\text{-}3}$. (For reference on side-chain couplings in thiophenes and pyrroles, cf. refs. [6] and [7].)

Indirectly, this gives evidence for the α-thiocyanation of unsubstituted pyrrole. If pyrrole is thiocyanated in the 3-position, it is to be expected that 2-methylpyrrole also will be thiocyanated in that position, since a methyl group in the 2-position of five-membered aromatics appears to activate the 3-position more than the 5-position in electrophilic substitution. It is known that although thiophene is nitrated in the 2-position, large amounts of the 2-methyl-3-nitro isomer are formed besides the 2-methyl-5-nitro isomer in the nitration of 2-methylthiophene [6].

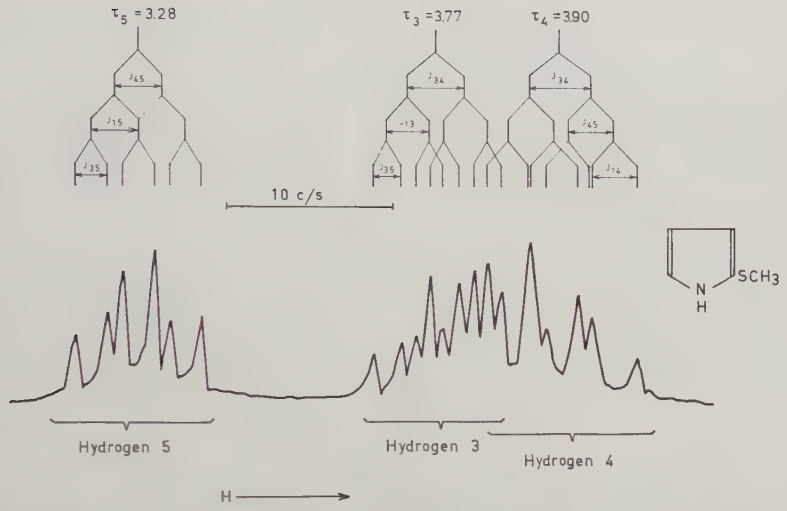


Fig. 4. Ring proton resonance spectrum at 40 Mc/s of 2-methylthiopyrrole in 46 % dioxane solution. The N–H band is not shown.

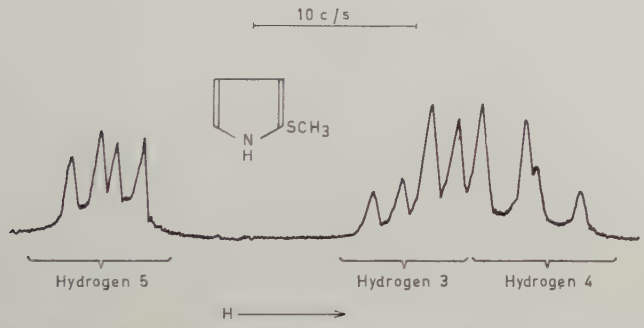


Fig. 5. Ring proton resonance spectrum at 40 Mc/s of 2-methylthiopyrrole in 46 % dioxane solution containing small amounts of piperidine. The N–H band is not shown.

Through the reaction of the thiocyanopyrrole with alkali and methyl iodide, Matteson and Snyder [2] obtained a methylthiopyrrole which they consequently assumed to be 3-methylthiopyrrole.

Repeating their experiment we obtained a compound which, because of its NMR-spectrum and because of chemical evidence (cf. below), was identified as 2-methylthiopyrrole. The complex spectrum of this compound (Fig. 4) was readily interpreted by making use of the information obtained from the simple NH-decoupled spectrum (Fig. 5), which consists of three bands of quartets at $\tau = 3.28$ ppm, $\tau = 3.77$ ppm and $\tau = 3.90$ ppm. The coupling constants have the values of 1.50 ± 0.10 c/s, 2.85 ± 0.10 c/s and 3.35 ± 0.10 c/s. The spectrum cannot be reconciled with that expected for the 3-isomer. Neither of the two higher field bands can be assigned to hydrogen 5, since this would lead to the unacceptably large value of 0.50 (or 0.63) ppm for the

upfield shift of this hydrogen (cf. $\delta_5 = +0.03$ ppm in 3-methylthio-thiophene [5]). Thus one would have to assign the lowest field band to hydrogen 5, in which case one would arrive at the equally unacceptable value of 3.35 c/s for J_{24}.

The spectrum is, however, in good agreement with that expected for the 2-isomer. The assignment in Fig. 5 is based on the values of the ring coupling constants, giving the values of $\delta_3 = -0.06$ ppm, $\delta_4 = +0.07$ ppm and $\delta_5 = +0.01$ ppm, for the shifts relative to pyrrole, which compare favourably with those of the thiophene analogue ($\delta_3 = +0.03$ ppm, $\delta_4 = +0.18$ ppm, $\delta_5 = +0.05$ ppm [5]).

We obtained the same methylthiopyrrole (IR-, NMR-evidence) through the reaction of pyrrolemagnesium iodide with dimethyl disulphide. In ether solution most of the starting material was recovered and only traces of the methylthio compound were obtained. By changing from ether to benzene, a 14 % yield of the methylthiopyrrole was obtained, together with some tarry products.

Although the question of the structure of the pyrrolemagnesium halides cannot be considered as completely solved (cf. refs. [9] and [10]). it has been shown that the reaction of the pyrrolemagnesium halides with a diversity of reagents such as acid chlorides, esters, carbon dioxide and halogens leads to the *α-isomers* when this position is free (for reviews, cf. refs. [9] and [10]). The only case where the formation of the β-isomer has been claimed is in the reaction between pyrrolemagnesium halides and alkyl halides [11, 12]. However, later authors have not been able to confirm this result [13, 14].

The same methylthiopyrrole was also obtained through the reaction of pyrrole with methyl sulphenyl chloride in ether at $-25°$ in the presence of anhydrous potassium carbonate. Even under these mild conditions, it was impossible to stop the reaction completely at the monosubstitution stage and the methylthiopyrrole was accompanied by pyrrole and a compound of b.p. 95°/1.5 mm Hg, which proved to be 2,5-dimethylthiopyrrole by elementary and NMR-spectral analyses.

This reaction illustrates the great reactivity of pyrrole towards electrophilic reagents. In the benzene series, Friedel–Crafts catalysts such as aluminium chloride have to be used in order to introduce the methylthio group by means of methylsulphenyl chloride [15]. However, it is well known that the Friedel–Crafts reaction proceeds with pyrroles even in the absence of catalyst, the entering group occupying the α-position [16].

It seems highly improbable that both the reaction of pyrrolemagnesium iodide with dimethyl disulphide and the reaction of pyrrole with methyl sulphenyl chloride lead to the 3-isomer. It is also very improbable that the rather low yields are due to the formation of both isomers, one of which subsequently decomposes completely. Actually, low yields are often obtained in pyrrole chemistry due to the known instability of pyrrole under certain reaction conditions.

Additional evidence showing that the methylthiopyrrole obtained in the 2-isomer is derived from the NMR-spectrum of the aldehyde prepared through Vilsmeier formylation of the methylthiopyrrole. This reaction is known to occur in pyrrole in the α-position [17].

The NMR-spectrum of the aldehyde (Figs. 6*a* and 6*b*) shows a sharp peak for the aldehydic hydrogen, proving that the other α-position is blocked with a substituent. It has been shown that 2-pyrrole- [7], 2-furan- [18] and 2-thiophenealdehydes [6, 18] having unsubstituted 5-positions show a characteristic coupling of 1 c/s to the hydrogen in this position. The ring coupling constant ($J = 3.80 \pm 0.10$ c/s) is of the magnitude characteristic for J_{34} in pyrroles and the chemical shift between

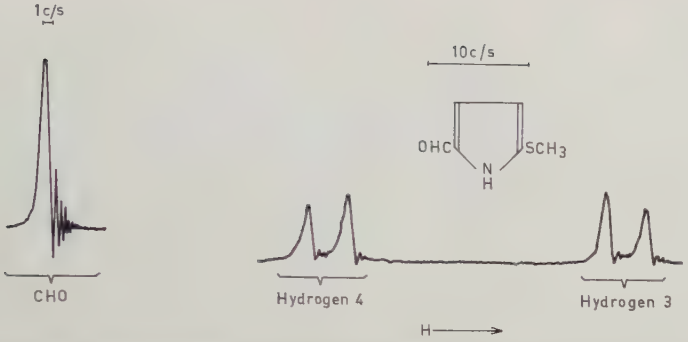


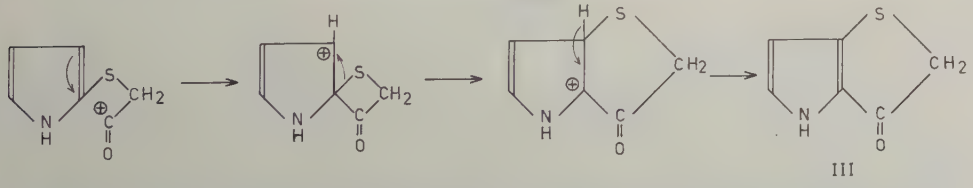
Fig. 6. NMR-spectrum at 40 Mc s of 2-methylthio-5-pyrrolealdehyde in 38 % dimethyl sulphoxide solution. In this solution the prototropy of the *N*-hydrogen is rapid enough to eliminate the couplings between the *N*-hydrogen and the *β*-hydrogens. (*a*) Aldehyde proton band; (*b*) the bands of hydrogen 3 and 4.

the ring hydrogens is also of the magnitude expected for 5-methylthio-2-pyrrolaldehyde.

Thus both NMR-spectra and chemical data show rather conclusively that the methylthiopyrrole obtained in three independent ways is the 2-isomer. The formation of 2-methylthiopyrrole from the thiocyanopyrrole involves a nucleophilic attack of an hydroxyl ion at the nitrile carbon followed by alkylation of the intermediate thiopyrrolate anion by the added methyl iodide. As none of these two processes involves a rearrangement, this gives additional evidence that also the thiocyanopyrrole is the 2-isomer. Likewise, no rearrangement can occur in the analogous preparation of the pyrrolylthioacetic acid and it follows from the foregoing that the compound described by Matteson and Snyder [2] as (3-pyrrolylthio)acetic acid (II) is the 2-isomer. However, the structure of 2H.3H-thieno[3,2-b]pyrrole-3-one (III) is proved beyond any doubt by Snyder *et al.* not only through desulphurization to 2-acetylpyrrole [2], but also by conversion to thieno[3,2-b]pyrrole [2], which has also been prepared starting from thiophenes [19].

Thus it is evident that a rearrangement must have occurred in the ring-closure with polyphosphoric acid at 120°, the oversight of which lead Matteson and Snyder [1, 2] to wrong conclusions about the structure of the thiocyano- and the methylthiopyrroles.

It is possible that the initially formed (2-pyrrolylthio)acetic acid first rearranges to the 3-isomer which then ring-closes to the 2-position to give III. An alternative path through a cyclic concerted mechanism is indicated schematically below.



Rearrangements during treatment with polyphosphoric acid, although not analogous to that found here, have been observed before. Banfield *et al.* [20] found that

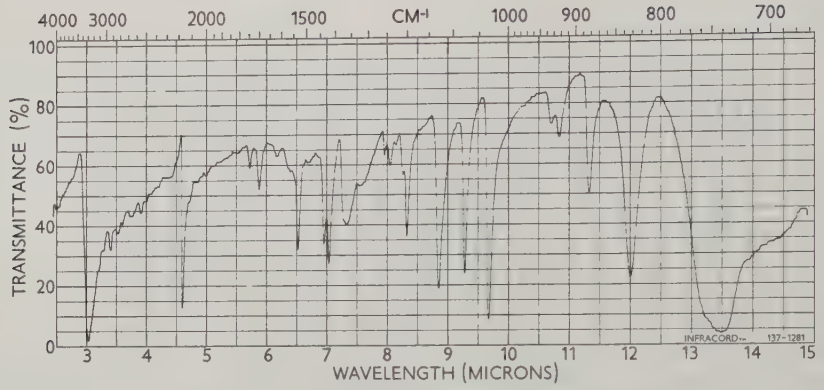


Fig. 7. IR-spectrum of 2-thiocyanopyrrole in KBr.

treatment of phenacyl phenyl sulphide with polyphosphoric acid gave 2-phenylthianaphthene instead of the expected 3-phenylthianaphthene. Similarly, phenyl phenacyl ether yields 2-phenylbenzofuran [21].

The present case is thus another example of the danger in applying the principle of minimum structural change in structure determination.

In an earlier study [22] when we had no reason to doubt the correctness of Matteson and Snyder's structure assignment of the monothiocyanopyrrole, we used it as a basis for the structure determination of the dithiocyanopyrrole obtained earlier by Söderbäck [23]. The NMR-evidence given there showed the dithiocyano compound to be symmetrically substituted, hence it follows from the present investigation that it is the 2,5-isomer, as originally suggested by Söderbäck [23], and not the 3,4-isomer [22].

We consider that the evidence presented in this paper conclusively shows that monothiocyanation of pyrrole occurs (as other electrophilic substitution reactions) in the α-position. As additional proof, we have arranged for an X-ray crystallographical investigation of the dithiocyanopyrrole, in order to definitely determine the structure of the thiocyanopyrroles, a question which is of importance not only for the theory of aromatic substitution of the π-excessive heteroaromatics, but also for establishing the degree of reliability of the NMR-method when use is made of the regularities observed in the spin couplings and the chemical shifts of aromatic ring systems.

Experimental

2-Thiocyanopyrrole

This was prepared according to both methods given by Matteson and Snyder [2]. We found the cupric thiocyanate method the more convenient. M.p. 41.5–43° (IR-spectrum, Fig. 7).

2-Methyl-5-thiocyanopyrrole

To 1.9 g (0.023 moles) of 2-methylpyrrole [24] in 25 ml of dry methanol at 0–5° was added with stirring 8.2 g (0.045 moles) of cupric thiocyanate [25]. After 50 min,

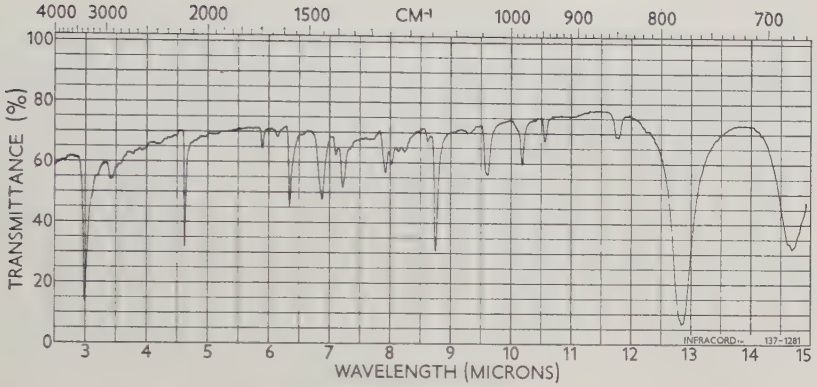


Fig. 8. IR-spectrum of 2-methyl-5-thiocyanopyrrole in KBr.

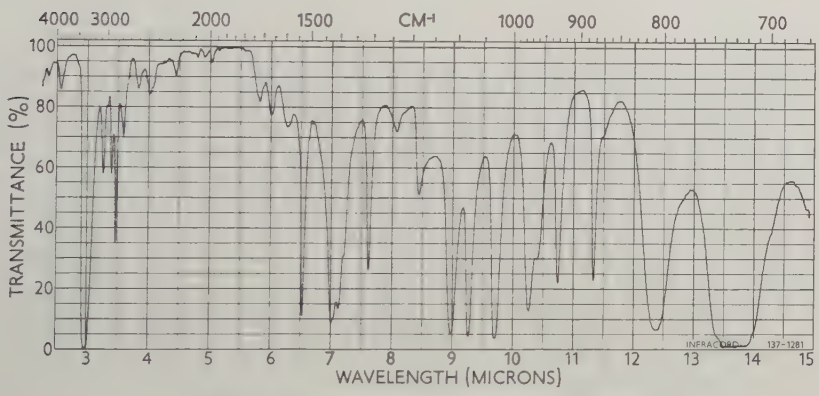


Fig. 9. IR-spectrum of 2-methylthiopyrrole.

the cuprous thiocyanate was filtered off and washed with 12 ml of methanol. The filtrate was poured onto 70 g of ice and 23 g of sodium chloride was added. The precipitate was filtered off and taken up in 50 ml of hot methylcyclohexane. On cooling 1.1 g (38 %) of pure 2-methyl-5-thiocyanopyrrole, m.p. 65.5–66°, crystallizing in long colourless flakes, was obtained (IR-spectrum, Fig. 8).

Anal. $C_6H_6N_2S$ (138.2)

Calc.	C 52.15	H 4.38	N 20.28	S 23.19
Found	C 52.22	H 4.39	N 20.21	S 22.86

2-Methylthiopyrrole, method I

This was prepared according to Matteson and Snyder [2]. The formation of a non-pyrrolic somewhat lower boiling impurity with characteristic peaks at 6.02 μ, 7.45 μ and 11.08 μ in its IR-spectrum and a band at $\tau = 6.4$ ppm in its NMR-spectrum neccessitated careful fractionation in order to isolate pure 2-methylthiopyrrole, b.p. 85–87°/15 mm Hg, $n_D^{20} = 1.5728$ (IR-spectrum, Fig. 9).

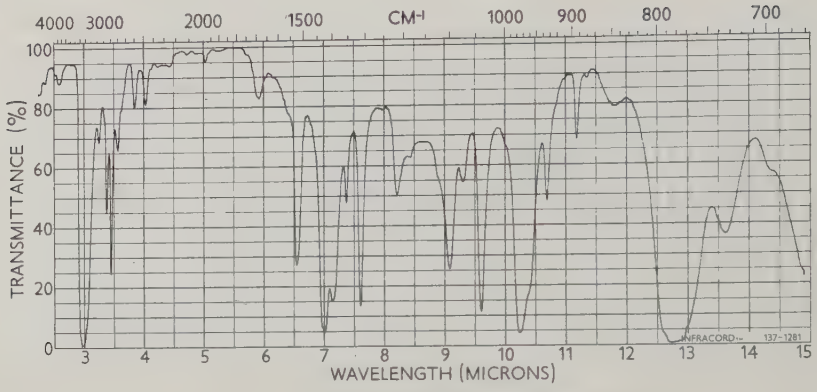


Fig. 10. IR-spectrum of 2,5-dimethylthiopyrrole.

2-Methylthiopyrrole, method II

To a solution of methyl magnesium iodide, prepared from 7.9 g (0.33 moles) of magnesium, 43.6 g (0.30 moles) of methyl iodide and 100 ml of ether, was added dropwise 20.1 g (0.30 moles) of pyrrole in 100 ml of ether. After refluxing for 30 min, most of the ether was distilled off and 150 ml of dry benzene was added simultaneously. 28.2 g (0.30 moles) of dimethyl disulphide was then added and the mixture refluxed for 18 h. The cooled mixture was then decomposed with ammonium chloride solution. The organic layer was separated, washed with water, dried over magnesium sulphate and fractionated. 4.3 g (21 %) of pyrrole, b.p. 33°/17 mm Hg (identified through its IR-spectrum), was recovered and 4.8 g (14 %) of 2-methylthiopyrrole, b.p. 87–90°/17 mm Hg, $n_D^{20} = 1.5730$, was obtained. Its IR- and NMR-spectrum was identical with those obtained in the preparation according to Matteson and Snyder [2]. An analytical sample was redistilled.

Anal. C_5H_7NS (113.2)

Calc.	C 53.06	H 6.23	N 12.38	S 28.33
Found	C 53.17	H 6.42	N 12.35	S 28.25

2-Methylthiopyrrole and 2,5-dimethylthiopyrrole, method III

To a stirred solution of 20.1 g (0.30 moles) of pyrrole in anhydrous ether and 45.5 g (0.33 moles) of anhydrous potassium carbonate was added dropwise under nitrogen at −25°, a solution of 24.8 g (0.30 moles) of methylsulphenyl chloride [26] in 200 ml of ether. After standing at −25° for an additional half-hour, the temperature was allowed to rise to 0° and 250 ml of water was added. The ether phase was separated, washed with water, dried over potassium carbonate and fractionated. 7.5 g of a fraction, b.p. 33–50°/17 mm Hg, consisting mainly of pyrrole (identified by its IR-spectrum) was recovered.

The second fraction (3.2 g), b.p. 50–115°/16 mm Hg, consisted mainly of 2-methylthiopyrrole. Refractionation gave 2.3 g (6 %) of pure 2-methylthiopyrrole, b.p. 85–87°/17 mm Hg, $n_D^{20} = 1.5728$, the IR-spectrum of which was identical with those of the other two preparations. A third fraction, 6.7 g (12 %), b.p. 115–135°/16 mm Hg, consisted mainly of 2,5-dimethylthiopyrrole, which after refractionation yielded

Table 2. Chemical shifts (ppm) and coupling constants (c/s) of pyrroles in dioxane solutions.

The shifts were obtained as f_i-values [4] and converted to τ-values [28] using τ (dioxane) = 6.430 ppm. The coupling constants were obtained from the observed splittings applying third-order perturbation correction [4], assuming like signs for all the couplings in the monosubstituted compounds. The uncertainties in the values obtained are ± 0.10 c/s.

ıbstituents	Concentration weight %	τ_3	τ_4	τ_5	Other shifts	J_{34}	J_{35}	J_{45}	Other couplings
CN	40	3.47	3.85	3.10	—	3.60	1.50	2.90	$J_{13}=2.45$; $J_{14}=2.40$; $J_{15}=2.85$
H_3, 5–SCN	27	4.13	3.56	—	—	3.55	—	—	$J_{CH_3-3}=0.80$; $J_{CH_3-4}=0.35$
CH_3	46	3.77	3.90	3.28	$\tau_{SCH_3}=7.75$	3.35	1.50	2.85	$J_{13}=2.60$; $J_{14}\approx J_{15}\approx \approx J_{45}$
CH_3, 5–CHO	20	3.78	3.12	—	$\tau_{SCH_3}=7.59$; $\tau_{CHO}=0.69$	3.80	—	—	
CH_3, 5–SCH_3	41	3.85	3.85	—	$\tau_{SCH_3}=7.77$				$J_{13}=J_{14}=2.60$

5.0 g (9 %) of pure 2,5-dimethylthiopyrrole, b.p. 95°/1.5 mm Hg, $n_D^{20}=1.6113$ (IR-spectrum, Fig. 10). Its NMR-spectrum in dioxane solution consists of three bands with relative intensities 1:2:6 due to the NH, the ring hydrogens and the side-chain hydrogens. In dioxane solution, the ring proton band is a doublet (2.6 c/s) which collapses into a singlet upon addition of small amounts of piperidine. The NMR-parameters are listed in Table 2.

Anal. $C_6H_9NS_2$ (159.3)

Calc.	C 45.24	H 5.70	N 8.80	S 40.26
Found	C 45.24	H 5.78	N 8.92	S 39.73

2-Methylthio-5-pyrrolealdehyde

To the stirred phosphorus oxychloride dimethyl formamide complex from 4.6 g (0.030 moles) of phosphorus oxychloride and 2.2 g (0.030 moles) of dimethyl

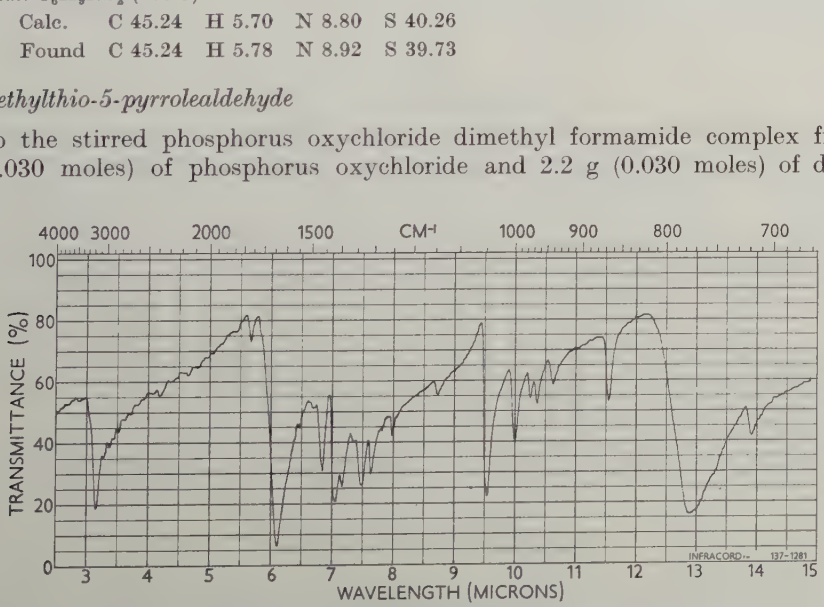


Fig. 11. IR-spectrum of 2-methylthio-5-pyrrolealdehyde in KBr.

formamide and 10 ml of ethylene chloride was added dropwise at 0° a solution of 3.1 g (0.027 moles) of 2-methylthiopyrrole in 10 ml of ethylene chloride. The reaction mixture was refluxed for 15 min. After cooling to room temperature, 12 g of sodium acetate in 30 ml of water was added and the mixture refluxed for 15 min. After cooling, the organic layer was separated and the aqueous phase extracted with ether. The combined organic phases were washed with sodium carbonate and water and dried over potassium carbonate. Removal of the solvent *in vacuo* yielded 3.7 g (87 %) of crude 2-methylthio-5-pyrrolealdehyde, m.p. 100–103°. Crystallization from petrol, (b.p. 80–100°) (charcoal) yielded 2.7 g of pure aldehyde, m.p. 105–106°, crystallizing in colourless needles. Further recrystallisation did not change the m.p. (IR-spectrum, Fig. 11).

Anal. C_6H_7NOS (141.2)

Calc.	C 51.04	H 4.99	N 9.92	S 22.71
Found	C 51.41	H 5.15	N 10.08	S 22.56

The NMR-spectra were obtained with a Varian Associates model V-4300-B high resolution spectrometer, operating at 40.00 Mc/s and a flux-stabilized 12 in. magnet equipped with shim-coils from the same company. The magnet sweep was calibrated with the modulation side-band technique using a 50.00 ± 0.05 c/s modulation frequency obtained from the power line. For the large shifts several side-bands, produced by increasing the modulation field [27], were used and the shifts were then measured on the recorder chart by interpolation.

The chemical shifts were obtained as f_1-values [4] relative to the solvents and converted to τ-values [28]. The τ-values of the different solvents were obtained from spectra of these solvents containing 2 % of tetra methyl silane.

The IR-spectra were recorded with a Perkin–Elmer Infracord Spectrophotometer.

SUMMARY

It is shown that thiocyanation of pyrrole occurs in the α-position in contrast to earlier statements, and that the methylthiopyrrole derived from the thiocyanopyrrole is the 2-isomer.

The structures of 2-thiocyanopyrrole and 2-methylthiopyrrole were proved by a study of the chemical shifts and the ring coupling constants in their NMR-spectra. Chemical evidence for the correctness of these structure assignments is derived from the fact that the same methylthiopyrrole is obtained both by reacting pyrrolemagnesium iodide with dimethyl disulphide and by reacting pyrrole with methylsulphenyl chloride, reactions which can be expected to give the α-isomer.

NMR-spectroscopy shows that the formylation product of methylthiopyrrole is 2-methylthio-5-pyrrolealdehyde, thus giving further proof that the methylthiopyrrole is the α-isomer.

2-Methylpyrrole is thiocyanated in the 5-position, as proved by NMR-spectroscopy.

The erroneous results obtained earlier are ascribed to a rearrangement in the cyclization of (2-pyrrolylthio)acetic acid with polyphosphoric acid to 2H,3H-thieno[3,2-b]pyrrole-3-one.

ACKNOWLEDGEMENTS

The authors wish to express their thanks to Prof. Arne Fredga and Prof. Kai Siegbahn for their interest in this work and for all facilities put at their disposal. Thanks are also due to Dr. E. Söderbäck for many interesting discussions on the chemistry of thiocyanogen and thiocyanates.

The elementary analyses were carried out at the Analytical Department of the Chemical Institute. Grants from the *Swedish Natural Science Research Council* to S.G. and from the *Swedish Technical Research Council* to R.A.H. are gratefully acknowledged.

Institute of Chemistry and Institute of Physics, University of Uppsala, March 1961.

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Tryckt den 21 september 1961

Uppsala 1961. Almqvist & Wiksells Boktryckeri AB

ARKIV FÖR KEMI Band 18 nr 10

Communicated 8 March 1961 by Hugo Theorell and Helmer Gustavson

An ellipsoid fluorimeter

By Sven Arrhenius

Introduction

In most fluorimeters the exciting light is collimated before passing the cuvette by a system of lenses. It necessitates either monochromatic light or achromatic lenses. Further, the accurate voltage stabilization for the light source and for the amplifier that is included in most commercial and laboratory-built fluorimeters also contributes to make them rather expensive. As there is a demand for simpler fluorimeters, especially for routine analyses, a fluorimeter will be described which can be built in an ordinary workshop.

For such an instrument, mirror optics is suitable. Before the construction in 1951 of the fluorimeter to be described here, mirrors have apparently not been used for macroscopic fluorimetry. In 1954 Theorell and Nygaard [5] described a fluorimeter, where the sample is illuminated inside a circular cylinder with walls, bottom and roof of aluminium. Such a mirror has, however, no focusing effect. Chance [1] put a fluorescent standard sample of plexiglass inside the mirror cylinder, and the photocurrent resulting from the fluorescence of this glass was used as a feed-back control for the light source. This highly sensitive but complicated instrument allowed him to study rapid and important muscle reactions.

In a mirror shaped as a rotation ellipsoid, light of all wave-lengths emerging from a source in one focus will be condensed in the sample placed around the other focus. In an ellipsoid cylinder with reflecting bottom, roof and ellipsoidal wall, practically the same illumination intensity will be reached. The disadvantages of this intense illumination are overheating and photo-chemical destruction. If the eccentricity of the ellipsoid is large, a good cooling may be arranged, and a shutter screening off the sensitive parts, except during measuring moments, can be given a convenient form. The photo-chemical destruction of the sample is unavoidable and causes the decrease in fluorescence known as "quenching". It can be eliminated by using a measuring technique of extrapolation back to unilluminated objects.

Monochromators may also be used in mirror fluorimeters. They are expensive without giving any appreciable advantages. In quantitative analyses it is sufficient to measure the whole amount of fluorescence. As many authors have shown, e.g. Franck and Rabinowitch [4] and Kasha [2, 3], the close coupling between rotation and vibration energy absorption and the electronic excitation allows an excitation using any wave-length within the absorption interval if enough energy is applied and the substances are sufficiently pure.

Principles

The principle of the fluorimeter can best be made evident from Fig. 2. It shows the high eccentricity of the elliptical mirror and its foci, where the light source and cuvette will be inserted. The solution is brought into this cylindrical cuvette to a height equal to the internal diameter of the cylinder. This is the ideal form for a fluorescent body. One focal axis of the mirror is not only the center line of the cuvette but also for a standard reference body of fluorescent plexiglass, formed as a flat cylinder and inserted close below the plane bottom of the cuvette from which it is screened off by a rubber sheet. As the cuvette and the light source along the other focal axis have diameters of more than a centimeter, their position within the ellipsoid does not become very critical. Also small deviations from an exact ellipsoidal form of the mirror will not appreciably diminish the focusing effect. This contributes fundamentally to the simplicity of the design. Thus, the apparatus is built with exchangeable mirrors of sheet-iron formed by their support.

The fluorescent light from the sample is taken out upwards through the meniscus of the solution, passes a diaphragm and a filter, and reaches a photo-electric cell (shown in Fig. 2) where the emf E_1 is generated. The fluorescent light from the plexiglass body is taken out downwards through a diaphragm with variable diameter and a filter, and produces an emf E_2. From the light source with the intensity I only the fraction r is reflected by the mirrors and also only the fraction a is transmitted by a filter in front of the fluorescent bodies. From the fluorescence of the sample F_1 (in F are included such factors as geometrical shape, concentrations, specific fluorescence, etc.) only the fraction b reaches the photo-electric cell, which has an emf yield of e_1. For the fluorescence of the reference body F_2 the part b reaching the photocell yields the effect e_2. The diaphragm is now varied until:

$$E_1 - E_2 = I a r F_1 b_1 e_1 - I a r F_2 b_2 e_2 = I a r (F_1 b_1 e_1 - F_2 b_2 e_2) = 0.$$

Thus at the balance point for the photo-currents it does not matter what intensity the lamp gives, or whether the mirrors or the filter in front of the sample have changed. This method for compensation also eliminates the thermo-currents in the junctions, and the photocells are both gently illuminated with the same amount of light equalizing any alteration; e_1 is approximatively $= e_2$. The filters in front of the photo-cells do not change with time.

The fluorescence F_1 and F_2 will change during illumination. This quenching is cumulative, and the fluorescence of a solution decreases incomparably more rapidly than that of a solid body. However, the alteration of the plexiglass must not be neglected. By measuring the fluorescence of standard solutions with known concentrations of the studied substance, the quenching of the reference body may be controlled. The unavoidable quenching of the sample must be observed and determined by measuring the fluorescence at short intervals after the first illumination instant, and extrapolating it back to zero time. By using an ordinary magnetophone recorder the observation intervals can be made as short as 5 seconds.

The accuracy of this method for compensation is dependent on the diaphragm. The drag makes it impossible to use iris diaphragms. A system of perforated plates is preferable. (In Fig. 2 one of the seven plates is shown in its position perpendicularly to the major axis of the mirror.) With an accuracy in the apertures of 0.01 mm (less than 1 %) and in the lateral setting of 1 mm, the diaphragm does not contribute to the experimental errors.

Description of the apparatus

The three main parts of the ellipsoid fluorimeter, the lower part of the lamp house (Fig. 2), the roof of this house, and the cuvette house (Figs. 2 and 3), are made of

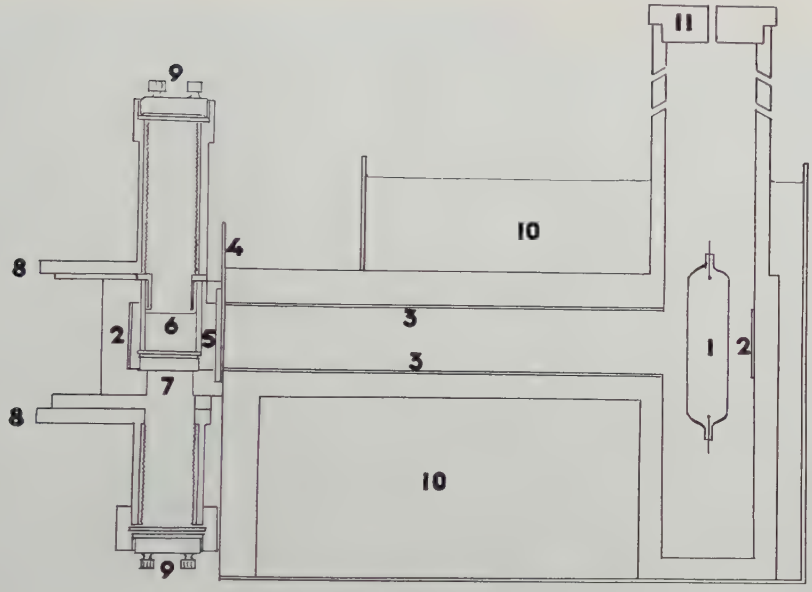

Fig. 1.

10 mm black oxide-coated cast aluminium. The parts of the lamp house are screwed together, and the joint tightened with rubber sponge and vaseline. As seen from the section picture (Fig. 1), the lamp house is surrounded by a water bath (10, Fig. 1). The light source (1, Fig. 1) is a mercury arc of 265 W requiring 220 V.[1] It is regulated by a variac. It hangs with its center line along the focal axis of the ellipsoid in a drilled plug of serpentine stone (11, Fig. 1) surrounded by the aluminium tubes, going out from the lamp house. Thus the lamp is electrically insulated, but is nevertheless connected to earth. The rest of the lamp house forms the major part of the ellipsoid cylindrical vessel for fixation of the mirrors. It is flat, 30 mm high, with a major axis of 262 mm, and a minor axis of 140 mm. Only 216 mm of the major axis is inside this house. The rest, 46 mm, belongs to the cuvette house. A 1 mm deep and broad elliptical groove for the mirror is cut in the bottom of the lamp house. The mirror (2, Fig. 1) is made of 1 mm polished stainless steel. On the bottom and roof of this lamp house flat mirrors (3, Fig. 1) of the same material may be inserted.

The cuvette house (Fig. 3) is not cooled. It is adjusted to the lamp house with locating pins and screwed to it with wing-nuts. In the joint between the lamp and cuvette houses is the shutter (4, Fig. 1), a 120×70 mm^2 plate of 0.5 mm aluminium. Immediately in front of the shutter, a tube for blowing cooling air opens into the house, and when the shutter is lifted the air blows over the first filter (5, Fig. 1). This is a Zeiss ultra-violet glass filter (UG3, Z8576), 120×40 mm^2, loosely fixed in a niche on the cuvette house.

With the focal axis as center, two holes are drilled in the cuvette house, one of 20 mm diameter in the bottom, and one with 30 mm diameter in the roof. A 4 mm

[1] The arc is a kernel of a "Luma hemsol" from AB Lumalampan, Stockholm.

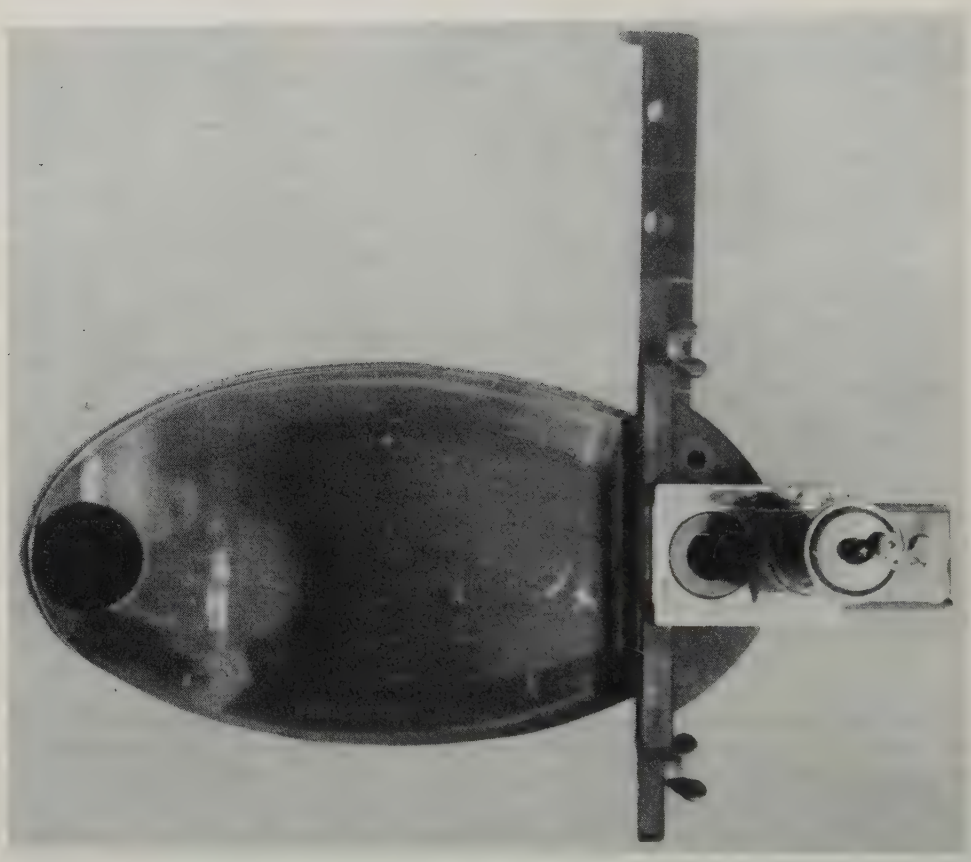

Fig. 2.

high cylinder of 22 mm diameter of fluorescent plexiglass[1] (7, Fig. 1), serving as a standard reference, is fixed inside the house above the bottom hole, and covered with a piece of rubber. On this rubber plate the cuvette (6, Fig. 1) is inserted through the upper hole. The cuvette is made of 40 mm quartz tube, inner diameter 23 mm, outer 26 mm. A tube diaphragm with an aperture of 17 mm and a length of 15.5 mm is settled down into the cuvette immediately before measurements and has no contact with the solution.

As shown in Fig. 2 the photocells (9, Fig. 1) are fixed in cylinders mounted on swallow-tailed slides (8, Fig. 1). These holders are electrically insulated from the photocells, and movable in the direction of the major axis. This movement permits the filling of the cuvette and the inserting of the diaphragms. The inside of the cylinders are covered with transversely riffled ebonite, in order to minimize false reflections. The length of the beams from the fluorescent samples to respective photocells are practically the same, 93 mm. Ordinary amateur photographic yellow filters (diameter 26 mm) are placed close to the photocells. The lower cylinder has a special

[1] From Allmänna Handelsbolaget, Stockholm.

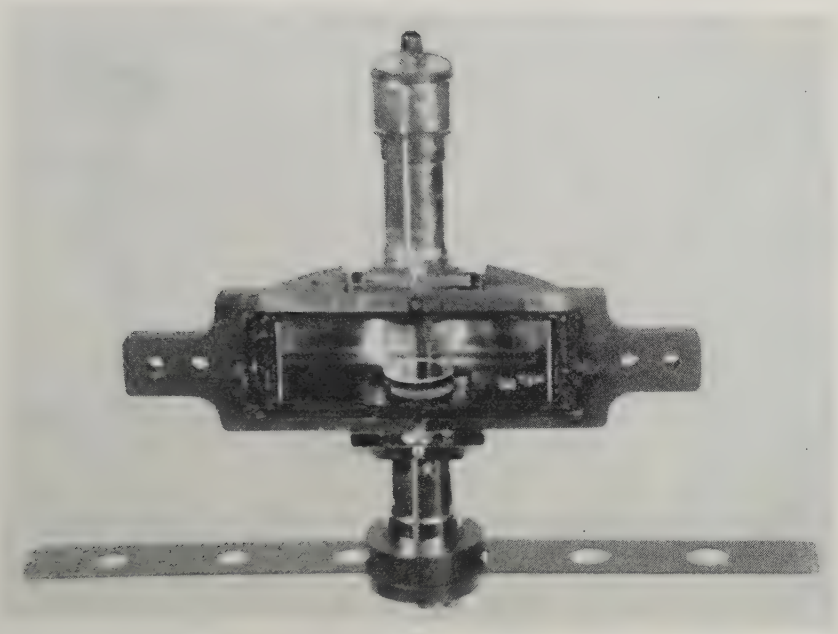

Fig. 3.

end piece of black nickel-plated brass with a 30×1 mm^2 cut for the perforated plates. These are made of 2 mm black nickel-plated brass, 300×30 mm^2. Each plate has six holes and carries in addition marks for setting with accurate diameter figures. The plate is pressed by a spring against the walls of the cut.

A slide giving shorter distance between sample and photocell has also been tried. Its advantage is greater sensitivity at the cost of accuracy, depending on the dispersed light. A multiflex galvanometer type Lange MG3 was used; it has a sensitivity of 2.6×10^{-9} amp/mm, and an inertia of about 5 seconds.

Method for measurements

When not in use the cuvette ought to be clean and dry. Before voltage is applied, cooling water and air must be running. The time for the light source to become stable is five minutes. The cuvette is filled with 10.0 ± 0.5 ml of solution. If the order of magnitude of the fluorescence is not known beforehand, the sensitivity of the galavanometer must be reduced, and the plate adjusted until the hole giving a deflection near zero has been found. This operation can be made very rapidly using a perforated plate for the preliminary survey. The exposed sample is spoiled by quenching and is discarded. A fresh sample is added to the cuvette, the galvanometer brought to its highest sensitivity, the recorder started, and all data such as concentrations, aperture, etc. read to it. On starting a stop-watch, the shutter is opened and the deflections are read at 5, 10 ... 30 seconds. Then the shutter is brought back rapidly in order not to expose the filter too long. Afterwards the values are

Table 1. Calibration of holes.

Diameter of hole in mm	Observed deflections Series 1	Series 2	Calculated deflections
1.0	0.9	1.0	0.9
1.5	1.7	2.0	2.1
2.0	3.4	3.8	3.8
2.4	5.2	5.4	5.4
2.8	7.2	7.5	7.4
3.2	9.4	9.7	9.7
3.6	12.3	12.7	12.2
3.9	14.3	14.6	14.4
4.2	16.5	16.7	16.7
4.5	19.7	19.6	19.1
4.8	22.1	21.8	21.8
5.0	23.8	23.7	23.6
5.1	25.0	24.8	24.6
5.2	25.6	25.2	25.6
5.4	27.7	27.4	27.6
5.6	30.3	29.9	29.6
5.8	32.1	31.6	31.8
6.0	35.1	34.6	34.0
6.0	34.8	34.6	34.0
6.3	37.5	37.4	37.5
6.6	41.4	40.8	41.2
6.9	42.5	44.7	45.0
7.2	49.5	48.8	49.0
7.5	53.6	53.1	53.1
7.5	53.1	52.8	53.1
8.0	60.5	59.5	60.5
8.5	68.5	67.0	68.3
9.0	76.3	74.3	76.5
9.5	87.0	84.5	85.3
10.0	94.8	91.9	94.5
10.0	94.0	91.5	94.5
11.0[a]	101.7	101.7	114.3
12.0[a]	131.1	127.7	136.1
13.0[a]	152.6	146.9	159.7
14.0[a]	175.2	169.5	185.2
15.0[a]	201.1	193.2	212.6
15.0[a]	198.9	194.4	212.6
16.0[a]	226.0	220.4	241.9
17.0[a]	248.6	236.2	273.1
18.0[a]	273.5	259.9	306.2
19.0[a]	282.5	270.1	341.1

[a] These values determined with decreased sensitivity of the galvanometer (0.1 × normal). Mean value (2.0 to 10.0) = 3.78. Standard deviation 0.04.

plotted from the magnetophone recorder, and extrapolated to zero time. The error in the extrapolation was found to be less than 0.3 mm, or about the accuracy in reading the galvanometer.

Control of the apparatus

The deflections were found to change with 1.19 ± 0.02 % per unit voltage change around 220 V. As variations of ± 5 volts are quite normal, an error of up to 12 %

Table 2. Fluorescence of fluorescein.

Content $\times 10^{-6}$ g/l	Hole diam. used	Deflection from zero	Corrected deflection	Deflection per gram $\times 10^{-8}$ [a]
0	0	4.1	0	—
1	2.4	0.6	2.3	2.30
2	2.8	1.0	4.8	2.40
4	3.6	0.7	9.7	2.43
6	4.2	1.1	14.9	2.48
8	4.2	5.1	18.9	2.36
8	4.5	2.6	19.0	2.38
8	4.8	0.6	19.8	2.48
10	5.2	1.2	24.5	2.45
12	5.6	1.0	27.7	2.31
14	6.0	2.4	34.8	2.49
16	6.6	0.5	40.5	2.53
18	6.9	0.3	44.4	2.47
20	7.2	0.6	49.0	2.45
22	7.5	2.1	54.9	2.49
25	8.0	1.9	62.6	2.50
28	8.5	0.5	69.5	2.48
30	8.5	5.2	74.2	2.47
32	9.0	3.6	81.5	2.55
35	9.5	0.8	88.1	2.52
37.5	10.0	−2.2	95.0	2.53
40	10.0	3.4	100.6	2.52
45	11.0	−3.2	115.2	2.56
50	11.0	7.4	125.8	2.52

Mean value 2.47 ± 0.01 mm/g $\times 10^{-8}$.
[a] Calculated from 10 ml solution pro sample.

results without stabilization or compensation. The temperature increase in the cuvette during exposure is 0.1°C/min (with filter UG3). This heat cannot disturb the measurements. Only damage to the mirror or the filtra situated close to the fluorescent bodies had any detectable effect.

Calibration of the perforated plates

The data of Table 1 were obtained in runs with the cuvette filled with water, varying the diaphragm between the reference and its photocell by changing the diameter of the holes (six measurements for each value). The values are reduced by the blind value obtained in shuttering off the fluorescence from the reference totally. The voltage supply was corrected for every second measurement to 220 V. The galvanometer deflections should be proportional to the square of the diameters. The deviations are probably caused by errors in the galvanometer.

Calibration with fluorescein

1.00 mg of fluorescein was dissolved in 5 ml of ethanol, and diluted to 1000 ml with a buffer solution of sodium carbonate–bicarbonate of pH = 9.0. In order to depress the quenching, this stock solution was diluted with boiled-out distilled water. The values of Table 2 are taken from experiments with all mirrors in the

fluorimeter and 93 mm distance from center of the sample to photocell. The galvanometer deflection for a solution containing 10^{-6} g/l is found to be 2.51 ± 0.01 mm. Considering the accuracy in reading to be 0.3 mm, it is thus possible to detect a concentration of 1.2×10^{-7} g/l $= 3.6 \times 10^{-10}$ *M* fluorescein. The content in the cuvette is then 3.6×10^{-12} moles.

With a Coleman fluorimeter using the filter combination B2 (corning 3889 + 5113) and PC9A (Corning 3385) 5×10^{-9} *M* fluorescein may be detected. For a Zeiss ZSN4 fluorimeter the same sensitivity was obtained with a filter combination with transmission maxima 436 mμ and 520 mμ.[1]

In order to study the effect of the flat mirrors, a run without them was made. It gave a mean deflection of 1.26 ± 0.01 mm for the concentration of 10^{-6} g/l. Thus, about half of the light is reflected by these mirrors. Extremely dilute solutions (from 1 to 20×10^{-7} g/l) have been examined with the fluorimeter after shortening the distance from the middle of the sample to the photocell down to 36 mm. A mean deflection of 21.0 ± 0.6 mm for 10^{-6} g/l was obtained. Thus it is feasible to determine as low concentrations as 4×10^{-11} *M* fluorescein.

SUMMARY

1 The advantages and disadvantages of using mirrors instead of lenses in concentrating the exciting light for fluorescence are discussed.

2. A fluorimeter with ellipsoidal cylindrical mirrors has been constructed in which the effect of heat has been eliminated.

3. In using an ordinary magnetophone recorder the errors due to quenching are eliminated by extrapolation to excitation time zero.

4. By the use of a standard reference for fluorescence in conjunction with fixed apertures, the fluctuation of the voltage is compensated without the use of stabilizers.

5. A sensitivity of 4×10^{-11} *M* fluorescein is reached without amplification of the photo-current or using photo-multipliers.

ACKNOWLEDGEMENTS

The author wishes to thank Mr. Ingvar Andersson for his excellent co-operation in the construction of the instrument and to Mr. Lennart Ström for technical assistance. The author also gratefully acknowledges helpful and informative discussions with Dr. Harry Svensson.

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[1] The author is greatly indebted to Dr. Lars-Göran Allgén for these values.

Tryckt den 21 september 1961

Uppsala 1961. Almqvist & Wiksells Boktryckeri AB

ARKIV FÖR KEMI Band 18 nr 11

Read 26 April 1961

The labelling of nucleic acids in tissue cells

By A. Forssberg, G. Dreyfus and G. Hevesy

The problem of the dynamic stability of the DNA molecule was studied in numerous investigations. The lines of attack chosen were the determination if and to what extent P^{32}, C^{14} or H^3 labelled precursors are incorporated into DNA of cells or tissues, and if and to what extent in DNA once incorporated P^{32}, C^{14} or H^3 atoms are released again.

In incorporation studies the relationship between the rate of labelling of DNA and the weight increase of the tissue or of its DNA has been computed during the observation period. Since the weight increase is often negligible in short-time experiments, many workers have used the comparison of the rates of isotope incorporation with the mitotic index. Mitotic index figures are, however, very low in adult animals and the values given by different authors often vary within fairly wide limits. Brues [1] estimated the mitotic index of resting rat liver to be 1×10^{-4} to 2×10^{-4}; Schultze and Oehlert, on the other hand, give the figure 4×10^{-4} [2]. Consequently the determination of the mean life-time of the tissue cells, based on incorporation data and mitotic index figures, becomes highly dependent on the reliability of the index figures scored in a certain strain and at a certain age of the animals.

The release of activity from the DNA is often referred to as "replacement". This term may imply an exchange of labelled DNA atoms against inactive ones, that is a metabolic activity of DNA. Alternatively, a loss of DNA label could occur concomitant with cell death. Whether some reutilization of nucleic acid breakdown products from dead cells takes place may be difficult to judge in general; experimental evidence for a certain reutilization has been given by Thompson [3]. Such a process would of course curtail the loss of tissue activity. If exchange and reutilization of labelled atoms can be chiefly excluded the method of studying the decrease of DNA activity would afford another method of surveying the mean life-time of cells. Complications may, however, arise when tissues are composed of cells with very different mitotic activities and life-times. Bone marrow, spleen, thymus and intestines have been frequently used in studies. These tissues contain very mobile cells, and a flow to or from the tissues of labelled cells may vitiate the results. The cells of the intestinal mucosa are rapidly renewed. Possibly the short life-times arrived at by many workers in such studies are rather due to changes taking place in the number and composition of mobile or short-lived cells and not to the main cell body of the tissues. Analyses of tissues therefore give, at best, over-all estimations of the DNA and cellular stability.

In spite of these and other limitations a survey of the literature makes it very probable that, with the exception of some special cases [4, 5, 6, 7], no atomic renewal

of the DNA molecule takes place [8, 9] and that DNA is only built up in connection with cell division and new formation of cells or with the additional synthesis of nuclear DNA in cases of polyploidy. For reasons which will be discussed below, we are inclined to believe that the determination of the release of once incorporated DNA label in experiments of long duration is a more trustworthy indication than analysis of its incorporation as far as the question of the DNA stability is concerned. In our experiments we have followed this line.

Methods and results

Groups of pregnant heterozygote white mice were given adenine-8–C^{14} (1 mC/mM) intraperitoneally three days before partus, 10 μC to each animal. The mice were caged separately in order to facilitate a division of each litter after the suckling period into two roughly equal groups in regard to number and sex. In series I 51 mice were sacrificed 30 days post partus (6 days after separation from the mothers), and a second group of 48 animals 116 days post partus. The brain, liver and spleen were secured, minced and made lipid-free through extraction with alcohol–ether. DNA was isolated following the procedure of Hammarsten [10]; moreover, RNA was isolated as well. In the second series the first group of each litter (26 animals) was sacrificed 40 days post partus, the second (28 animals) at 130 days. In this series we isolated the mixed nucleic acids.

In regard to the possible methodological errors by the isolation of nucleic acids we tried to minimize these by running all the operations in each series of experiments concomitantly. The isolated DNA (RNA), as judged from the UV-absorption curves, was nearly of the same purity in all samples. On the other hand, the yields, when compared to the dry weights of the organs, indicated slight deviations from proportionality.

Activity measurements were performed on infinite thick layers of samples to statistical significance within an error of $\pm 5\,\%$. No traces of acid-soluble activity was found in the tissues. Corrections for the dilution of activity due to the increase of DNA (RNA) in the organs of the second group of each series is subject to some error because of the variations in the yield of nucleic acids. We have therefore also applied an alternative correction, namely for the dry weight increase of the lipid-free organs in the second group. Implicit in the latter procedure is the assumption of a proportional increase of organ weight and nucleic acids in the second groups. The validity of this assumption is impaired if polyploidy occurs in older tissues; this is possibly the case on a minor scale in the liver. However, the two different ways of calculating the results mainly agreed; we believe this fact to substantiate the results.

The daily percentage loss of activity has thus been computed (Table 1), based both on the weight increase of DNA and RNA respectively (corr. 1), and on the dry weight increase of the tissues (corr. 2).

Discussion

The rate of incorporation of labelled precursors into the DNA of cells and tissues was in many instances found to proceed at a similar rate as the formation of new cells. This implies that DNA synthesis takes place as a preparation for mitosis and

Table 1. Daily percentage loss in purine C^{14} activity of DNA and RNA.

Series I: first group sacrificed 30 days, second group 116 days post partus; Series II: 40 days and 130 days respectively post partus. Corr. 1, spec. activities corrected for weight increase of DNA (RNA) in group II. Corr. 2, for weight increase of dry tissue.

Fraction	Organ		
	Liver	Spleen	Brain
Series I			
DNA:			
Corr. 1	0.57	0.42	0.14
Corr. 2	0.30	0.42	0.28
Mean	0.44	0.42	0.21
RNA:			
Corr. 1	0.78	0.57	0.62
Corr. 2	0.48	0.41	0.52
Mean	0.63	0.49	0.57
Series II			
DNA + RNA:			
Corr. 1	0.23	0.23	0.20
Corr. 2	0.12	0.31	0.16
Mean	0.18	0.27	0.18

that no significant loss of once DNA bound activity except for that caused by occasional cell death does occur. In regenerating liver, for example, cell division was found to go hand in hand with incorporation of P^{32} into DNA by Kihara and Sibatani [11], by Takagi and Potter [12], by Daoust *et al.* [13] and others. Regenerating liver following hepatectomy may be said to constitute an exceptional case, since the predominating process of new cell formation in the regeneration period, and thus the incorporation of P^{32} into the DNA, may mask a possible small scale loss of once synthesized DNA.

In these studies the incorporation rate was assayed in a fairly short time interval following the isotope administration. In long-term experiments similar studies were performed by Fresco and Bendich [14] on the fate of the once incorporated DNA label from adenine–N^{15} and glycine-2–C^{14}, given to partially hepatectomized almost full-grown rats. The first analysis on the liver DNA activity was performed after the lapse of 28 days and further analyses were made at intervals up to 121 days. No acid-soluble labelled compounds could be traced at 28 days. After a correction for a slight liver weight increase it appeared that very little activity had been lost in the observation period. The authors calculated a mean life-time of 1070 days for the label, which is nearly equivalent to the life-time of the animals. Probably the small loss of C^{14}-atoms in this case may be entirely accounted for by cell death and replacement with new unlabelled cells. This figure is by a factor of 10 or more higher than those commonly given for liver, based on experiments of short duration. The results imply that when there is a considerable lapse of time before the first analyses, the label from particularly short-lived cells disappears and that the apparent life-time of the bulk of the tissue cells consequently increases.

Also in rapidly growing normal liver of young rats Swick [15] found the daily

DNA synthesis to be in conformity with the view that DNA is formed as a preparation for mitosis only, as computed from a quantitative equality between the synthesis of the DNA and the weight increase of the organ. The liver of the adult rat grows slowly; the same author gives a figure of 7 per cent in about two months. In the adult liver most of the daily synthesized DNA is probably made available for the replacement of dead cells. This replacement was calculated as somewhat less than 1 per cent per day, a figure which is of the same order as that of the mitotic activity, for which various authors have given values ranging from 0.15 to 1 per cent. Swick also arrives at the conclusion that the loss of DNA is mainly if not entirely accounted for by cell death and degradation of DNA and that "intracellular turnover" is a rare or non-existent process.

Several authors have reported incorporation rates of DNA label of the order of about 0.5–1 per cent per day. The incorporation of P^{32} into the DNA of the liver of fully grown rats was found by one of the present writers and Ottesen [16] to be 4 per cent in the course of 4 days and a value of 0.1 per cent was observed in experiments taking 2 hours [17]. Similar values for the extent of P^{32} incorporation were found by a number of other investigators as well [18, 19, 20, 21]. These figures were also corroborated in autoradiographic experiments of Messier *et al.* [22]. According to these authors 0.2 per cent of the liver cells of mice, given thymidine–H^3, were labelled in the course of 8 hours.

In experiments of very short duration, in which either the rate of incorporation or the rate of loss of once incorporated DNA activity were computed, the results arrived at are sometimes difficult to interpret. Gerber *et al.* [23] gave rats three consecutive injections of thymidine–H^3 and analysed muscle, liver, kidney, intestines, spleen and thymus 1–30 days after the last injection. They noted some urinary activity two days after the last injection, indicating the presence of minor amounts of labelled precursor material. Moreover, the pool of active precursors in the various tissues may vary greatly in such experiments. It is interesting to note that the authors found a considerable loss of DNA activity from intestines, spleen and thymus during the first days followed by a slower decay. Their interpretation was that the DNA of these organs contain two "components" with different average life-times. The rapidly decaying component was lacking in liver, kidney and muscle. Nygaard and Potter [24] also found a rapid decrease of the DNA–thymidine label during the first three days in the above-mentioned organs, as well as in bone marrow. Still another observation of Ottesen [25] corraborates the early activity decay in some tissues. He counted the percentage of nuclei with two or more tracks in smears of rats spleen, labelled in DNA with C^{14}–adenine. The activity loss between day one and ten amounted to about 85 per cent as compared to a further decrease of only 55 per cent in the following 50 days.

Based on the rates of activity decay the mean life-time of the tissue label has been calculated in these short-time experiments. For example, Gerber [23] gives 40 days for spleen and 30 days for intestines, both values applying to the "slow" DNA component. The "fast" component decayed at a rate of a few days only. From Bendich's data in an earlier publication [26] one arrives at 25 days for liver cells, 10 days for spleen and 5 days for intestines. In experiments of short duration, in which a small fraction of the cells get labelled, the apparent labelling of the whole tissue may be partly due to the existence of short-lived or mobile cells. The intestinal mucosa, for example, is build up of cells which are renewed rapidly. A flow of labelled cells, particularly leucocytes, to and from organs like spleen has also been proven [27].

The apparent life span of the DNA and the cell seems therefore to depend on the tissues analyzed and the time schedule of analysis in each instance.

The daily percentage loss of DNA (RNA) activity in our material (Table 1) does not differ appreciably when comparing the organs in each series. The greater stability of the DNA brain label which is suggested from the Series I determinations is not corroborated by the other brain analyses. In view of the experimental errors connected with the analyses, as well as with the corrections for DNA (RNA) or tissue weight increase, the figures, taken together, strengthen the view that all the organs are characterized by a rather similar activity loss when the early activity changes have vanished. This similarity suggests either the presence of cells of a roughly similar life span, or alternatively one or several DNA (RNA) fractions of a similar stability in these tissues. Actually some tissue cells seems to retain the DNA label for a considerable time. According to Leblond [28] autoradiographic staining from thymidine–H^3 may be as intense in the nuclei six months after the labelling as after three days.

The mean life span of the DNA (RNA) label can be computed from the figures in Table 1. In Series I one arrives at about 115 days for liver and spleen and about twice that figure for brain. As said above the experimental errors hardly allow us to postulate a greater stability of the brain tissue DNA. An RNA fraction of a high stability was found in the organs, which shows a decrease in activity not much larger than that of DNA. The half-life of that RNA label amounts to 80–100 days.

In the second series the first analysis was postponed until 40 days post partus, whereas the time interval to the sacrifice of the second group in each litter remained the same as in the first series. The mixed nucleic acids showed an activity decay which is essentially smaller than in the first series, probably because some RNA fraction of comparatively short life span had decayed to a greater extent before the first analysis. It is noteworthy that the apparent life-time of the label of the cells as calculated from our decay figures seems to increase with the prolongation of time elapsing between the labelling of the foetus and the first analyses.

SUMMARY

The dynamic stability of DNA (RNA) in the liver, spleen and brain of mice was assayed in the offspring of females, given C^{14}–adenine in the late stage of pregnancy. The first group in each litter was analyzed 30–40 days post partus, the second one about 80-90 days later. The three organs showed an essentially similar activity loss in that time interval. A labelled RNA fraction was found to decay at a rate which only little exceeded that of the DNA. The implications of these studies are discussed in relation to the results of other workers.

ACKNOWLEDGEMENTS

The generous support of the Swedish Natural Science Research Council and of the Alice and Knut Wallenberg Foundation is gratefully acknowledged.

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Tryckt den 21 september 1961

Uppsala 1961. Almqvist & Wiksells Boktryckeri AB

ARKIV FÖR KEMI Band 18 nr 12

Communicated 25 January 1961 by Arne Fredga and Gunnar Blix

Mass spectrometric studies

VII. Methyl esters of α,β-unsaturated long-chain acids. On the structure of C_{27}-phthienoic acid

By Ragnar Ryhage, Stina Ställberg-Stenhagen and Einar Stenhagen

With 12 figures in the text

The structure of the mycolipenic and phthienoic acids isolated from tubercle bacilli has been the subject of conflicting reports. It has been suggested by Bailey, Polgar and Robinson [1] (cf. ref. [2]) that C_{27}-phthienoic acid [3] is identical with mycolipenic acid for which, on the basis of degradation work performed on what appears to have been rather impure material, they proposed a 2,4,6-trimethyl-2-enoic acid structure. Cason *et al.* [4] did not accept this structure for C_{27}-phthienoic acid and objected to the name "racemic C_{27}-phthienoic acid" given by Asselineau *et al.* to *trans*-DL-*erythro*-2,4,6-trimethyltetracosen-2-oic acid in a preliminary report [5] of synthetic work [6].

A mass spectrometric study showed, however, that one sample of methyl C_{27}-phthienoate kindly provided by Professor Cason gave a mass spectrum very similar to that of the synthetic *trans*-ester [2]. The mass spectrum of the methyl C_{27}-phthianoate prepared by catalytic hydrogenation of this sample was identical with that of synthetic 2,4,6-trimethyltetracosanoate (mixture of diastereoisomers) obtained by catalytic hydrogenation of *cis*-DL-*erythro*-2,4,6-trimethyltetracosen-2-oate. Another sample of methyl C_{27}-phthianoate also provided by Professor Cason gave a mass spectrum showing a number of peaks not present in the mass spectra of the two saturated esters just mentioned [2]. It was later found that the last-mentioned sample could be separated into two components by means of gas chromatography. The major component gave a mass spectrum identical with that of the first sample of methyl C_{27}-phthianoate, whereas the minor component gave an entirely different mass spectrum which it has so far not been possible to interpret. Lack of material has prevented the examination of the corresponding alcohol which might have provided a clue to the structure. A gas chromatographic study indicated that the small differences noted between the mass spectra of methyl C_{27}-phthienoate and synthetic *trans*-DL-*erythro*-2,4,6-trimethyltetracosen-2-oate [2] were due to the presence of about 5 per cent of methyl DL-*erythro*-3,5-dimethyltricosanoate in the synthetic sample. When allowance is made for this there is no significant difference between the mass

Table 1. Material used.

Compound	Melting point, °C	Ref.
Methyl *cis*-octadecen-2-oate[a,b]	[c]	
Methyl *trans*-octadecen-2-oate[a,b]	32.5–33.1 [d]	
Methyl *trans*-2-methyleicosen-2-oate[b,g]		[16]
(−)-Ethyl *trans*-2,4D-dimethylheneicosen-2-oate	23.6–24.0 7 [e]	[7]
(−)-Methyl *cis*-2,4L,21,21-tetramethyl-docosen-2-oate	25.5–25.8	[8]
(+)-Methyl *trans*-2,4L,21,21-tetramethyl-docosen-2-oate	27.1–27.5	[8]
(−)-Methyl *trans*-2,5D-dimethylheneicosen-2-oate	14.0 8.0– 8.5 [e]	[7]
Methyl *cis*-DL-*erythro*-2,4,6-trimethyl-tetracosen-2-oate	35.2–36.6 16.3–16.6 [e]	[2]
Methyl *trans*-DL-*erythro*-2,4,6-trimethyl-tetracosen-2-oate[f]	30.5–31.5 14.3–14.7 [e]	[2]
Methyl C_{27}-phthienoate[g,h]	30 –32.5 13 –15 [e]	[3]

[a] Prepared by means of methanol and sulphuric acid from a sample of the acid obtained from Dr. L. Crombie, University College, London.
[b] Purified by gas chromatography.
[c] Melting point of acid 50.5–51.0°C.
[d] Melting point of acid 57.4–57.8°C.
[e] Dimorphism.
[f] A gas chromatographic examination showed that the original sample contained approximately 5 per cent of methyl DL-*erythro*-3,5-dimethyltricosanoate.
[g] This ester was obtained from Professor James Cason of the University of California.
[h] The sample represented material that had been recovered from spectroscopic work.

spectrum of natural methyl C_{27}-phthienoate and that of the synthetic *trans*-ester. The results thus indicate that the conclusions of Cason *et al.* [4] are invalidated by the presence of two components in the material used for the oxidative degradation work described in ref. [4], and they provide strong evidence for the 2,4,6-trimethyl-substituted structure of C_{27}-phthienoic acid. The presence of a 2,4,6-trimethyl-substituted C_{27}-acid in lipids from tubercle bacilli has recently been confirmed by Murray [6], who used a novel degradation technique involving oxidative degradation by means of potassium permanganate and gas chromatographic separation and identification of the products.

In the present communication the mass spectra of all α,β-unsaturated long-chain esters that have been available to us (Table 1) are described.

Mass spectrometry. The apparatus and the technique used have been described previously [9, 10, 11]. The temperature of the intake system was kept at 200°C and the energy of the electrons was 70 eV. Available intensity data are given in Table 2.

Results

The mass spectra of the methyl esters of *cis*- and *trans*-octadecen-2-oic acids are shown in Figs. 1 and 2. The two spectra were run in immediate succession under identical conditions and we therefore believe that the differences between them are largely due to the geometrical isomerism. A characteristic feature of the spectra is

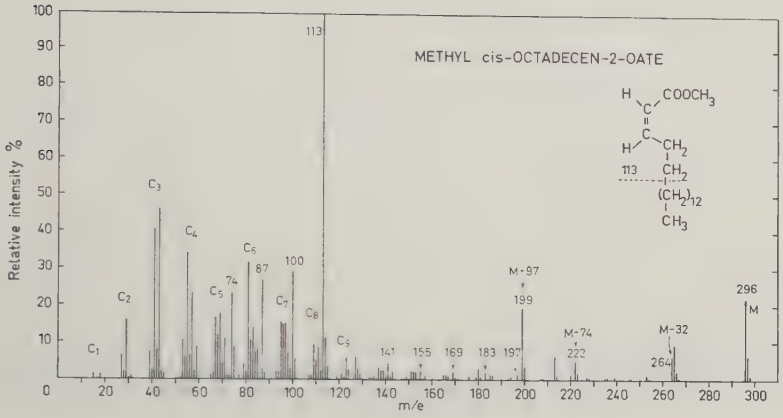


Fig. 1.

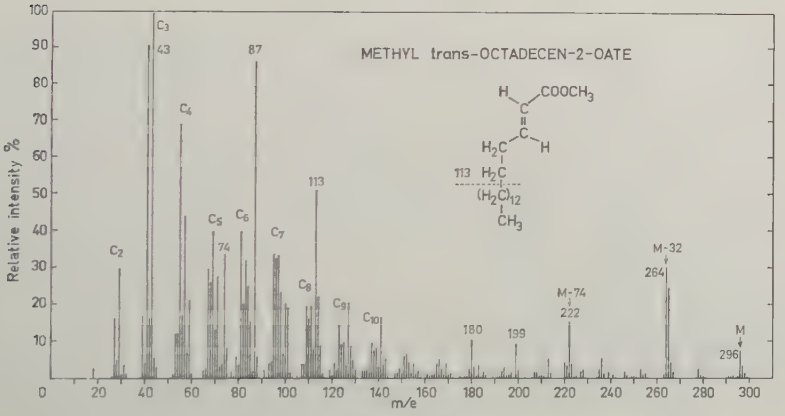
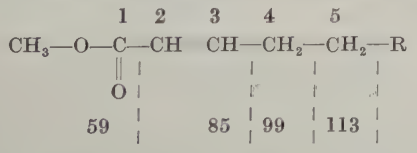


Fig. 2.

the occurrence of a high peak at *m/e* 113, presumably due to ions formed by 5,6-cleavage with retention of the double bond in its original position.



The methoxycarbonyl type peaks at *m/e* 74 and 87 must be due to ions formed by 2,3- and 3,4-cleavage respectively after the double bond has been displaced from its original position. In the intermediate mass range there appears a series of peaks at *m/e* 141, 155, 169, 183, 197, ..., which seem likely to be due to methoxycarbonyl-type ions containing the double bond. The contribution to these peaks from alkyl type ions is probably small. The normal series found for saturated esters (*m/e* 143, 157, 171, 185, 199, 213, ...) is also present and particularly those at *m/e* 199 and 213 are

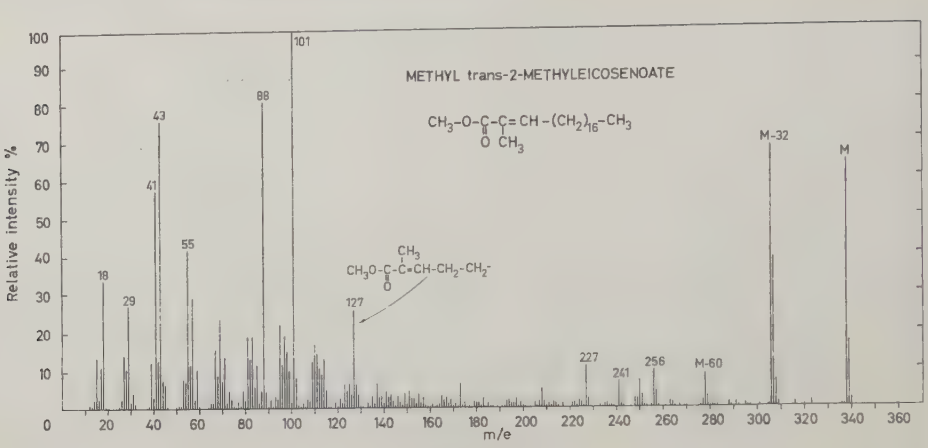


Fig. 3.

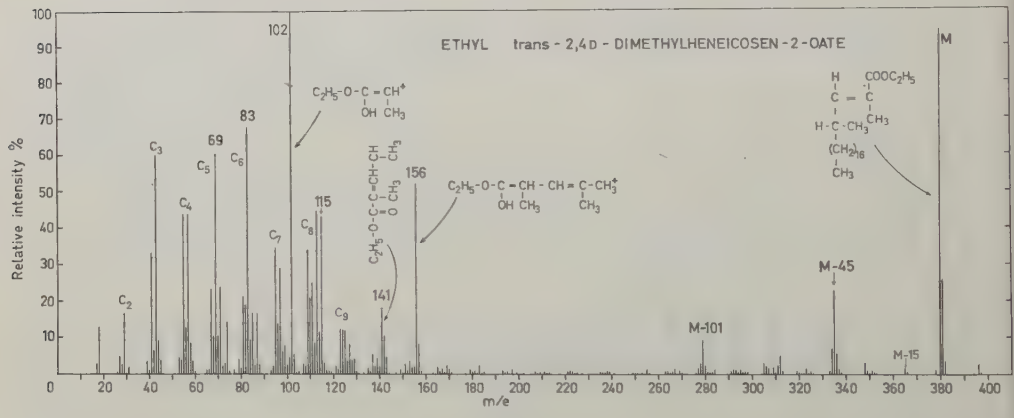


Fig. 4.

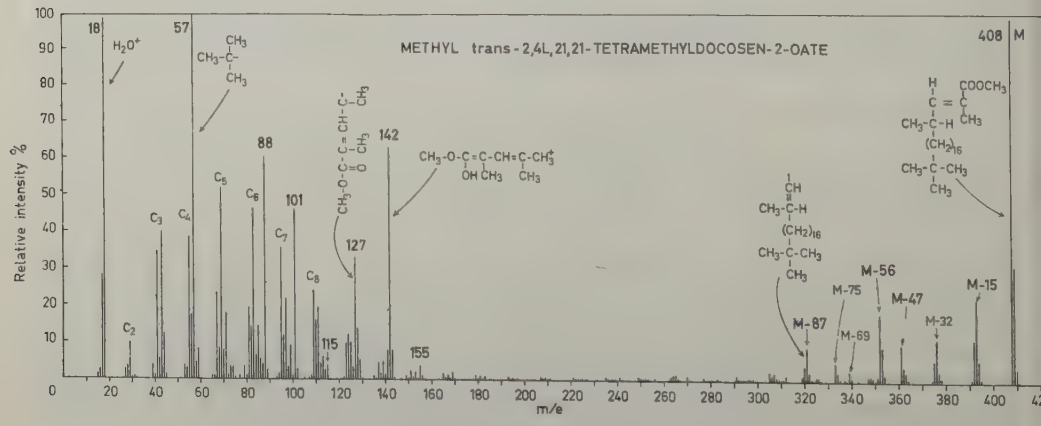


Fig. 5.

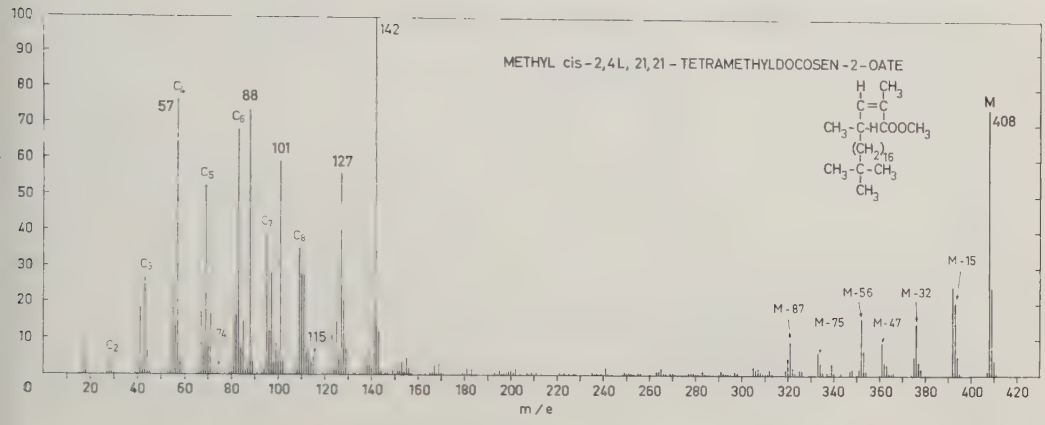


Fig. 6.

very much stronger than their companions at *m/e* 197 and 211 respectively. The presence of two series in this manner suggests that the two mechanisms found in the case of saturated esters [12, 13] also operate here, the saturated fragments being formed by the elimination of a part of the chain containing the double bond. In the high mass range there appear peaks at *m/e* 264 (= *M* − 32), 222 (= *M* − 74), and 180 (= *M* − 116) characteristic of methyl esters of mono-ethenoid C_{18} acids [14]. The mechanism of the formation of the last-mentioned ion is at present unknown, but the appearance of a peak at *m/e* = *M* − 116 is characteristic for methyl esters of mono-ethenoid acids [11]. In methyl 9,10-dideuterooleate[1] the corresponding peak occurs at *m/e* 182 (= *M* − 116) showing that the fragment contains the double bond.

The mass spectrum of methyl *trans*-2-methyleicosenoate (Fig. 3) shows high peaks at *m/e* 88 and 101 (base peak) in the same manner as saturated 2-methyl-substituted methyl esters. It is thus evident that both 2,3- and 3,4-cleavage are preceded by a double-bond shift. The fairly high peak at *m/e* 127 and the low peak at *m/e* 129, on the other hand, show that the methoxycarbonyl fragment arising from 5,6-cleavage contains the double bond. The peaks due to the higher methoxycarbonyl fragments are low and form no distinct pattern. The ions of *m/e* 227 and 241 appear to be formed by the elimination of part of the chain (carbon atoms 2–8 and 2–9 respectively). The high peak at *m/e* = *M*–32 is also found in the spectrum of Fig. 3.

The α,β-unsaturated 2,4-dimethyl-substituted methyl esters whose mass spectra are reproduced in Figs. 5–6 show peaks at *m/e* 88 and 101, and the analogous ethyl ester (Fig. 4) shows peaks at *m/e* 102 and 115, as do the saturated counterparts. The two methyl esters show a very characteristic peak at *m/e* 142 and the ethyl ester (Fig. 4) shows an analogous peak at *m/e* 156 due to ions formed by 5,6-cleavage and rearrangement of one hydrogen atom from the fragment lost. A possible structure of the ion is indicated in Fig. 4. Simple 4,5-cleavage with retention of the double bond gives ions of *m/e* 127. For the ethyl ester (Fig. 4) the corresponding ions have *m/e* 141. The mass spectra of the two methyl esters containing an ω-*tert*-butyl group (Figs. 5 and 6) show a very high peak at *m/e* 57 due to *tert*-butyl ions, and a smaller

[1] Ng. Dinh-Nguyen, unpublished data.

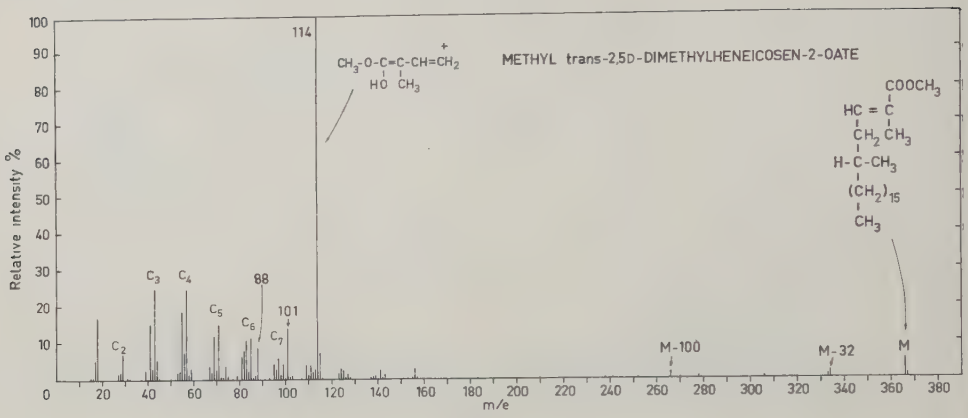


Fig. 7.

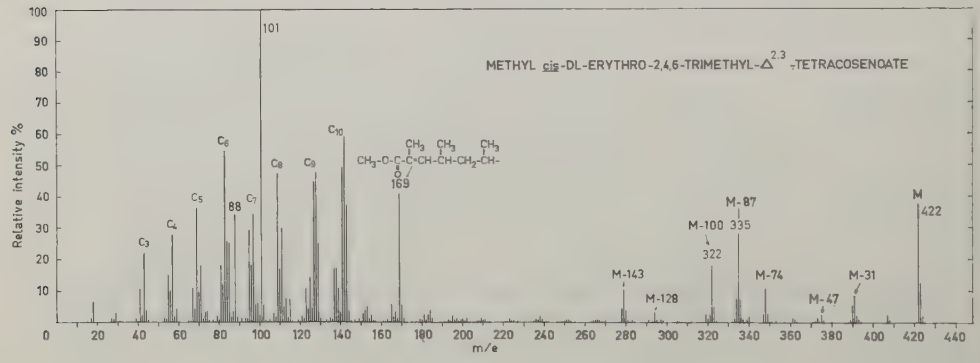


Fig. 8.

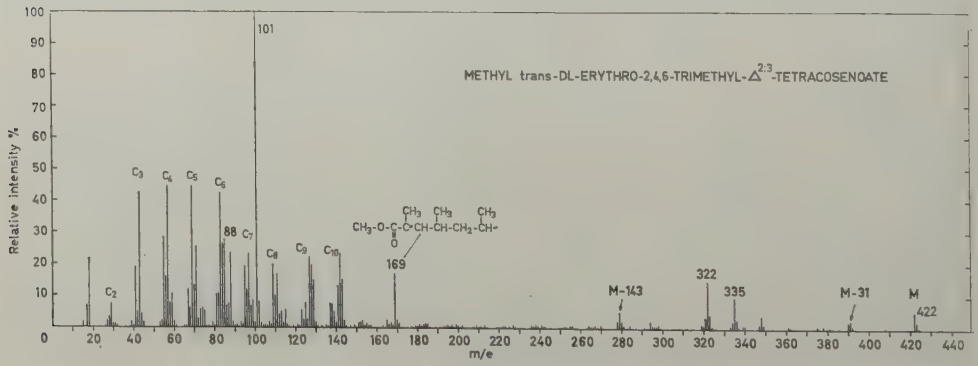


Fig. 9.

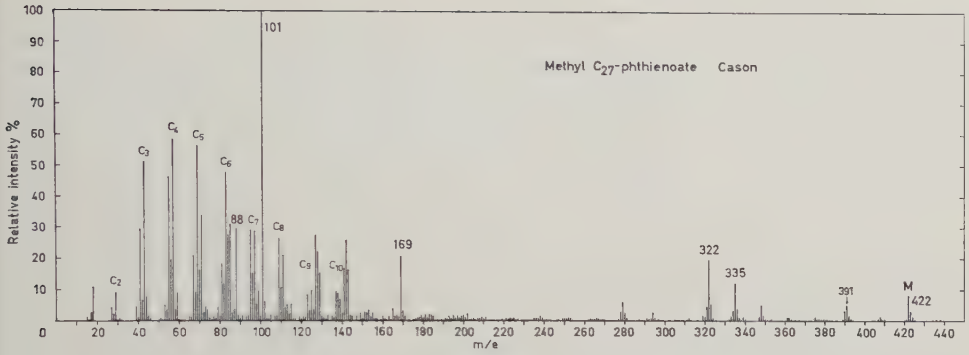


Fig. 10.

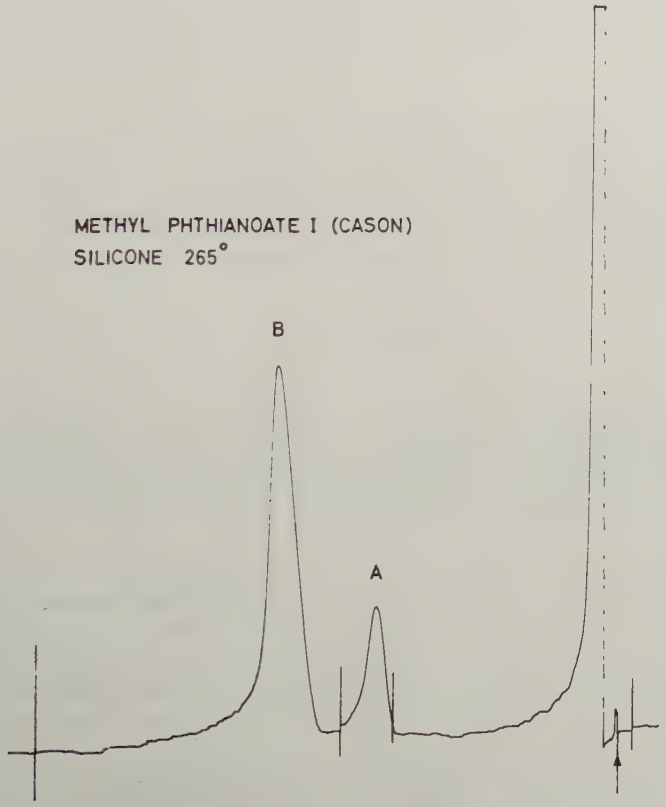


Fig. 11. Gas chromatographic separation of "methyl C_{27}-phthianoate I".

peak in the high mass range at $m/e = M-56$, similar to saturated *ω-tert*-butyl-substituted esters [15]. Peaks due to ions formed with loss of 15 and 16 mass units are also present. These appear to be due to ions formed with loss of a methyl group and a molecule of methane respectively from the molecule-ion.

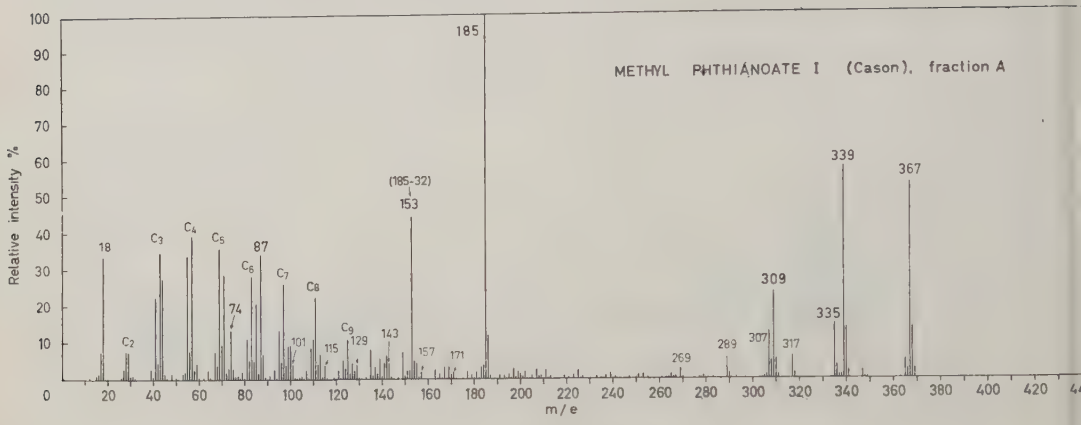


Fig. 12 a.

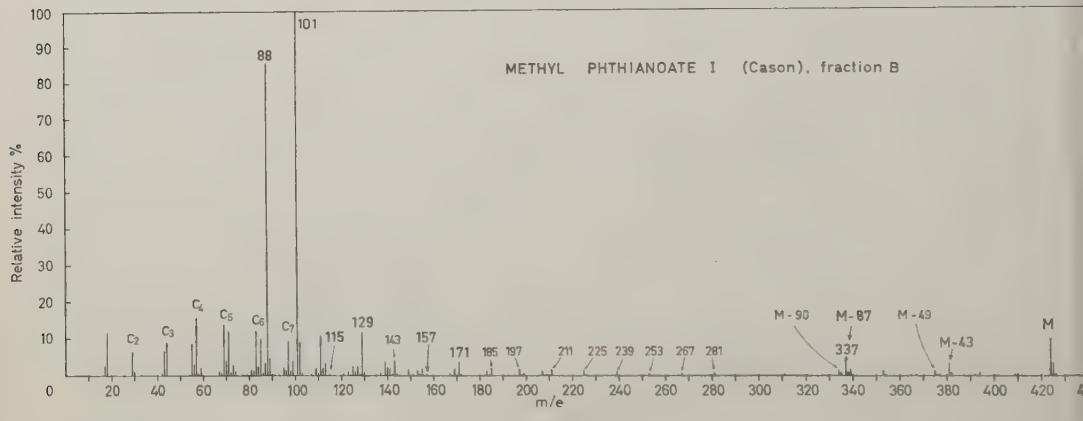


Fig. 12 b.

The mass spectrum of methyl *trans*-2,5D-dimethylheneicosen-2-oate (Fig. 7) is dominated by the peak at *m/e* 114. The ion responsible for this peak is formed by 4,5-cleavage with rearrangement of one hydrogen atom, and a possible structure with conjugated double bonds is indicated in Fig. 7. Like the 2- and the 2,4-dimethyl-substituted methyl esters high methoxycarbonyl type peaks are present at *m/e* 88 and 101.

The methyl *cis*- and *trans*-2,4,6-trimethyltetracosen-2-oates also show very high peaks at *m/e* 88 and 101 (base peak). A characteristic peak appears at *m/e* 169 (Figs. 8 and 9). The ions of *m/e* 169 appear to be formed by simple 6,7-cleavage of the chain. The *cis*- and *trans*-isomers differ mainly in the relative heights of the peaks at *m/e* 332 (= *M*–100) and 335 (= *M*–87), the ions of *m/e* = *M*–87 being formed in greater number in the case of the *cis*-ester. The mass spectrum of the *trans*-ester (Fig. 9) shows peaks due to the presence in the sample of approximately 5 per cent methyl 3,5-dimethyltricosanoate (as determined by gas chromatography).

This gives rise to, *inter alia*, the peak at *m/e* 74 as well as the molecule-ion peak at *m/e* 396 in the spectra reproduced by Ahlquist *et al.* [2]. The mass spectrum of the 3,5-dimethyl-substituted ester is given in ref. [15]. When the contribution due to this contamination is removed the spectrum of the *trans*-ester becomes undistinguishable from that of methyl C_{27}-phthienoate (Fig. 10).

The mass spectra thus furnish a direct proof of the presence of a *trans*-double bond as well as the presence of methyl side chains at positions 2, 4, and 6 in the sample of methyl C_{27}-phthienoate studied.

The gas chromatogram showing the separation of "methyl C_{27}-phthianoate I" into two components is shown in Fig. 11 and the mass spectra of the two components are given in Fig. 12. It has not yet been possible to obtain a satisfactory interpretation of the mass spectrum of component A. The spectrum of component B is identical with that of the saturated ester obtained by the catalytic hydrogenation of the sample of methyl C_{27}-phthienoate [2]. The mass spectra of the two samples of saturated methyl C_{27}-phthianoate as originally received together with that of synthetic methyl 2,4,6-trimethyltetracosanoate have been published previously [2].

SUMMARY

A mass spectrometric study has been carried out on long-chain α,β-unsaturated esters of different types. The results obtained with natural methyl C_{27}-phthienoate show that this ester has a *trans*-2,4,6-trimethyl-2-enoate structure. Gas chromatographic and mass spectrometric studies of two different samples of methyl C_{27}-phthianoate showed the presence of two components in one of the samples, a fact that seems to explain the conflicting reports in the literature regarding the structure of C_{27}-phthienoic acid.

ACKNOWLEDGEMENTS

We are indebted to Dr. L. Crombie, University College, London, Professor James Cason, University of California, Berkeley, and Mr. Ng. Dinh-Nguyen, University of Göteborg, for material used, and to Mrs. Marianne Forsberg and Mrs. Betty Ull for assistance in the treatment of mass spectrometric data. The financial support from *Statens Naturvetenskapliga Forskningsråd*, *Statens Medicinska Forskningsråd* and *Statens Tekniska Forskningsråd* is gratefully acknowledged.

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Tryckt den 21 september 1961

Uppsala 1961. Almqvist & Wiksells Boktryckeri AB

	Methyl cis-octadecen-2-oate	Methyl trans-octadecen-2-oate	Methyl trans-2-methyleicosenoate	Ethyl trans-2, 4D--dimethyltheneicosen-2-oate	Methyl trans-2, 4L, 21, 21--tetramethyldocosen-2-oate	Methyl cis-2, 4L, 21, 21--tetramethyldocosen-2-oate	Methyl trans-2, 5D--dimethyltheneicosen-2-oate	Methyl phthianoate I (Cason), fraction A	Methyl phthianoate I (Cason), fraction B	
	A	B	C	D	E	F	G	H	I	
M	296	296	338	380	408	408	366	...	...	
m/e										m/e
12	...	...	1.1	...	0.2	...	0.2	0.5	...	12
13	...	...	0.8	...	...	...	...	...	...	13
14	...	...	2.3	0.1	0.2	...	...	0.2	0.2	14
15	1.7	1.0	13	0.4	1.7	0.5	0.9	1.1	0.3	15
16	0.3	...	2.6	0.4	2.8	0.4	0.8	1.6	0.4	16
17	0.6	...	11	3.2	28	2.3	5.6	7.8	2.7	17
18	1.7	2.6	34	13	99	9.4	17	33	12	18
19	...	...	0.6	...	0.3	...	...	0.4	...	19
20	...	...	...	...	...	...	...	...	...	20
21	...	...	...	...	...	...	...	...	...	21
22	...	...	...	...	...	...	...	...	...	22
23	...	...	...	...	...	...	...	...	...	23
24	...	...	...	...	...	...	...	...	...	24
25	...	...	0.7	...	...	...	...	...	...	25
26	0.4	0.7	2.7	0.9	0.5	0.2	0.3	0.6	...	26
27	6.8	16	14	5.2	3.1	1.2	1.7	3.0	...	27
28	2.4	5.2	10	3.0	4.0	1.6	2.3	7.9	0.3	28
29	16	30	27	17	10	4.6	7.4	7.8	6.8	29
30	0.7	1.4	2.0	0.6	0.8	0.7	0.4	1.0	1.2	30
31	1.2	3.7	4.2	2.3	1.2	0.4	0.8	1.1	...	31
32	0.4	1.5	1.4	...	0.7	0.2	0.6	0.6	0.3	32
33	0.2	0.3	0.3	...	...	...	...	...	0.4	33
34	...	...	...	...	...	...	...	...	...	34
35	...	...	...	...	...	...	...	...	...	35
36	...	...	...	...	...	...	...	...	...	36
37	...	0.3	0.5	0.2	0.2	...	0.5	0.2	...	37
38	0.3	0.6	0.9	0.3	0.3	...	0.2	0.3	...	38
39	7.9	17	12	3.9	4.3	1.5	2.8	2.6	0.3	39
40	2.8	4.4	3.2	1.4	1.5	0.6	0.5	0.8	0.3	40
41	41	91	57	33	35	18	15	22	...	41
42	8.3	16	12	6.8	5.9	2.9	3.3	4.7	0.8	42
43	46	100	76	60	40	28	25	35	6.9	43
44	2.5	5.8	7.7	9.4	12	6.9	5.7	27	9.0	44
45	1.9	2.9	6.5	4.1	1.8	1.1	0.9	1.6	0.3	45
46	0.3	0.4	0.7	0.6	0.8	...	...	0.5	...	46
47	...	...	...	...	...	...	...	0.2	...	47
48	...	...	...	...	...	...	...	...	...	48
49	...	...	...	...	...	...	...	...	...	49
50	0.2	0.3	0.7	0.4	0.4	...	0.6	0.5	...	50
51	0.6	0.6	1.1	0.5	0.5	0.2	0.2	0.4	...	51
52	0.7	1.3	0.9	0.4	0.3	0.2	0.5	0.3	...	52
53	11	12	7.9	4.9	4.3	2.1	2.3	1.7	0.3	53
54	6.1	12	7.3	4.2	3.4	2.1	2.5	2.0	0.5	54
55	34	69	42	44	39	26	19.2	34	8.7	55
56	7.1	17	11	13	18	14	7.8	7.8	3.1	56
57	23	44	29	44	100	77	25	39	16	57
58	2.5	7.3	2.6	8.9	5.1	3.9	1.8	2.6	0.8	58
59	9.1	21.6	10	4.0	8.7	6.3	3.4	4.6	2.4	59
60	0.4	1.5	0.7	1.2	0.5	0.4	0.3	0.7	0.3	60
61	...	0.3	0.3	0.4	...	...	...	0.3	...	61
62	...	...	...	...	...	...	...	0.6	...	62
63	...	...	0.4	0.2	...	...	0.3	0.2	...	63
64	0.2	0.4	0.5	0.3	...	...	0.2	2.4	0.2	64
65	1.5	2.5	1.7	1.5	1.4	0.9	0.4	0.6	...	65
66	2.1	2.8	1.6	1.6	1.4	0.7	0.3	0.4	...	66
67	17	30	15	23	24	17	4.0	7.7	1.3	67
68	12	26	8.0	10	8.8	7.3	2.2	3.8	0.9	68
69	18	40	23	60	52	52	12	36	14	69
70	4.7	13	7.4	10	8.3	8.1	3.0	9.8	4.3	70
71	11	28	13	24	18	17	15	29	12	71
72	1.4	3.0	1.7	2.5	1.7	1.7	1.0	2.1	1.0	72
73	1.4	4.2	4.9	3.4	3.8	0.4	1.0	3.1	3.0	73
74	23	34	2.7	14	3.9	2.0	4.0	13	1.3	74
75	9.1	8.6	0.9	1.2	1.0	0.5	1.3	2.8	0.3	75
76	0.4	0.7	0.5	0.2	0.2	...	0.2	1.0	...	76
77	1.5	2.1	2.2	1.7	1.5	0.6	0.6	1.1	0.4	77
78	0.5	0.9	0.9	0.6	0.6	0.3	0.3	0.6	0.4	78
79	4.5	6.2	5.0	4.5	4.0	3.2	1.5	2.2	0.4	79

m/e	A	B	C	D	E	F	G	H	I	m/e
80	2.5	4.0	2.3	2.0	1.5	1.1	0.4	0.6	0.2	80
81	32	40	19	21	19	21	6.7	11	1.7	81
82	11	20	13	19	14	16	8.3	5.4	1.3	82
83	14	32	19	68	47	68	11	29	12	83
84	7.8	25	5.9	9.6	6.8	7.7	2.5	5.2	2.5	84
85	8.7	15	11	17	14	15	11	21	10	85
86	0.8	2.0	1.6	2.7	6.1	1.3	0.9	1.8	0.8	86
87	27	86	4.8	17	4.5	3.8	1.5	34	3.4	87
88	2.1	6.2	81	3.0	61	74	9.0	7.2	86	88
89	0.3	0.7	4.7	0.7	3.1	3.9	0.5	0.8	5.0	89
90	...	0.3	0.6	0.2	0.4	0.4	...	0.2	0.6	90
91	1.1	2.1	2.5	1.6	0.5	1.0	0.5	1.2	0.5	91
92	0.2	0.7	0.8	0.4	0.3	0.3	0.2	0.4	...	92
93	2.3	4.3	3.4	1.3	1.0	3.2	0.8	2.7	0.5	93
94	2.3	4.8	2.7	2.6	1.9	1.7	0.4	0.5	0.2	94
95	16	33	22	35	36	39	4.7	13	2.5	95
96	15	33	11	14	12	12	3.0	5.0	1.6	96
97	15	33	19	29	22	28	6.2	26	9.4	97
98	7.5	23	15	6.5	3.7	3.7	1.7	3.9	1.5	98
99	3.2	7.1	10	8.2	9.5	9.0	4.7	9.3	4.1	99
100	29	20	1.9	2.8	1.3	4.0	0.7	9.5	0.4	100
101	6.0	19	100	5.0	46	59	14	4.3	100	101
102	0.7	1.9	8.2	100	3.2	3.9	1.0	0.6	9.0	102
103	...	0.7	1.1	5.9	0.4	0.4	1.3	0.4	0.9	103
104	0.2	0.4	0.6	0.9	0.2	...	1.1	0.9	0.3	104
105	0.5	1.1	1.8	1.1	0.8	0.8	0.3	1.1	0.4	105
106	...	0.6	0.7	0.4	0.2	...	...	0.4	0.2	106
107	1.5	4.2	2.5	3.2	0.8	3.1	0.6	2.7	0.6	107
108	1.4	4.1	2.1	2.5	1.5	1.7	0.4	0.6	0.2	108
109	9.2	19	12	34	24	35	4.3	8.8	2.2	109
110	5.6	16	17	21	16	28	1.5	11	0.8	110
111	8.9	19	14	25	20	28	4.1	22	11	111
112	2.7	8.1	10	8.9	4.7	6.4	2.2	4.4	2.1	112
113	100	51	9.1	45	6.4	7.3	3.0	7.2	3.4	113
114	11	22	13	11	2.9	3.7	100	0.9	0.3	114
115	3.6	9.1	5.1	43	4.2	5.5	7.6	3.9	0.7	115
116	0.6	1.6	0.7	3.6	0.5	0.6	0.8	0.6	...	116
117	0.2	0.5	0.6	1.4	0.3	0.2	...	0.4	0.2	117
118	0.1	0.4	0.3	1.4	...	...	...	0.2	...	118
119	1.0	2.6	1.9	1.0	0.6	0.8	0.2	0.8	0.2	119
120	0.4	1.1	0.7	0.5	0.3	0.3	...	0.3	...	120
121	1.7	4.3	2.7	2.5	0.6	2.3	0.4	2.6	0.6	121
122	1.1	2.5	1.5	1.7	1.0	1.2	0.3	0.7	0.2	122
123	6.3	14	7.2	12	10	13	1.8	5.5	1.3	123
124	2.9	9.4	4.9	12	12	1.7	3.3	3.2	0.5	124
125	...	10	7.0	12	10	15	2.7	11	2.5	125
126	0.4	3.7	3.4	4.0	3.6	4.4	0.9	1.7	1.2	126
127	6.4	20	26	8.6	33	56	1.8	4.7	2.7	127
128	3.0	9.0	6.5	4.2	14	20	0.8	2.4	0.6	128
129	1.8	5.3	3.9	4.6	5.7	7.5	0.4	4.3	12	129
130	0.6	2.0	1.2	1.2	0.7	0.8	...	0.7	1.1	130
131	0.2	0.5	0.7	0.7	0.3	0.2	...	0.4	0.3	131
132	...	0.3	0.4	0.3	...	1.0	...	0.2	...	132
133	0.9	2.2	1.7	0.9	0.5	0.8	...	0.8	0.3	133
134	0.7	2.3	1.0	0.4	0.2	0.2	...	0.3	...	134
135	1.2	3.4	3.3	2.0	1.3	2.0	0.3	8.3	0.6	135
136	0.6	1.7	1.4	1.1	0.7	0.8	0.2	1.2	0.2	136
137	3.6	9.7	6.9	6.0	4.8	6.2	0.8	3.5	0.9	137
138	2.9	7.9	3.1	2.5	1.9	2.8	1.0	1.7	0.4	138
139	2.8	8.6	3.5	4.8	5.3	8.1	1.2	5.9	4.0	139
140	0.9	3.3	1.6	2.4	1.7	2.2	0.5	1.0	2.0	140
141	4.8	16	4.6	18	8.3	15	2.9	4.7	2.1	141
142	1.1	3.4	3.1	10	63	100	0.8	6.8	0.6	142
143	2.3	5.9	3.8	5.1	8.3	12.2	1.8	4.6	4.2	143
144	0.5	0.9	2.1	0.7	0.8	1.2	0.3	0.9	0.7	144
145	...	0.4	0.8	0.4	0.3	0.3	...	0.5	0.4	145
146	...	0.2	3.4	0.2	...	1.0	...	0.2	...	146
147	0.5	1.6	1.8	0.9	0.5	0.9	...	0.8	0.2	147
148	0.5	1.8	1.1	0.4	0.2	0.3	...	0.3	...	148
149	0.5	1.8	4.2	1.7	1.4	1.7	0.6	7.8	1.7	149
150	0.5	1.5	1.2	0.8	0.5	0.5	...	1.2	0.3	150
151	2.3	6.3	4.8	3.2	2.6	3.3	0.6	2.5	0.4	151
152	2.4	6.9	2.8	1.3	1.0	1.2	0.3	2.6	0.3	152
153	2.0	4.6	2.8	4.0	2.4	3.9	0.6	44	1.3	153
154	0.6	1.6	1.3	2.2	0.7	0.9	0.3	5.3	0.4	154
155	2.5	4.1	4.1	2.4	4.0	5.0	0.8	4.7	1.8	155
156	0.6	1.4	1.5	52	1.4	2.2	3.3	0.9	0.3	156
157	1.1	2.3	3.1	8.7	0.5	0.7	0.8	2.3	0.4	157
158	0.3	0.6	0.9	1.2	...	0.2	...	0.4	...	158
159	...	0.3	0.7	0.3	...	...	...	0.3	0.2	159

m/e	A	B	C	D	E	F	G	H	I	m/e
160	...	...	0.5	...	...	...	...	0.2	...	160
161	0.4	1.4	1.2	0.5	0.4	0.5	...	0.3	...	161
162	0.2	0.8	0.5	0.3	0.2	0.2	...	...	...	162
163	0.4	1.2	1.0	0.9	0.6	0.7	...	2.8	0.3	163
164	0.4	1.4	1.2	0.5	0.3	0.3	...	0.5	...	164
165	1.4	4.2	3.5	2.4	1.9	2.1	0.3	1.7	0.3	165
166	1.5	5.4	2.4	1.2	0.8	0.9	0.3	0.6	0.2	166
167	1.2	2.8	3.0	1.9	1.7	2.9	0.4	3.3	0.7	167
168	0.4	1.0	1.4	0.9	0.7	0.7	0.3	0.5	0.2	168
169	2.3	4.2	2.5	2.8	2.3	3.5	0.6	3.5	1.8	169
170	0.7	1.2	0.8	1.8	0.6	0.9	0.2	1.6	0.3	170
171	0.7	1.7	1.3	0.9	0.5	0.6	0.2	2.0	3.5	171
172	0.3	0.5	0.6	0.3	...	0.2	...	0.4	0.5	172
173	...	0.2	6.9	0.3	...	...	...	0.2	0.2	173
174	...	...	0.9	0.3	...	...	...	...	...	174
175	0.3	0.9	1.8	...	0.3	0.3	...	0.4	...	175
176	1.1	0.4	0.5	...	...	...	...	0.2	...	176
177	0.2	0.8	0.6	0.6	0.4	0.4	...	2.2	0.3	177
178	0.2	0.8	0.5	0.5	0.3	0.2	...	0.5	...	178
179	0.9	2.8	1.8	1.7	1.4	1.3	0.2	1.4	0.2	179
180	2.9	10	1.7	1.0	0.6	0.7	0.2	0.6	...	180
181	0.9	3.0	1.8	1.3	1.2	2.1	0.3	2.2	0.3	181
182	0.3	0.9	0.9	0.7	0.5	0.6	0.2	0.6	0.2	182
183	2.1	3.1	2.7	2.7	1.2	1.9	0.5	3.3	1.3	183
184	0.4	0.8	0.6	0.7	0.4	0.4	...	4.1	0.4	184
185	1.5	2.1	1.6	1.0	0.5	0.6	...	100	2.0	185
186	1.4	1.0	0.7	0.3	...	0.2	...	12	0.3	186
187	0.2	0.3	0.7	0.3	...	...	...	1.3	...	187
188	...	...	0.4	...	...	...	...	0.2	...	188
189	0.2	0.7	0.6	0.3	0.2	0.3	...	0.4	...	189
190	...	0.3	...	0.2	...	...	...	0.2	...	190
191	...	0.6	0.6	0.5	0.4	0.3	...	1.5	0.3	191
192	0.2	0.8	0.7	0.3	0.3	0.2	...	0.4	...	192
193	0.6	2.2	2.1	1.4	1.1	1.0	0.2	1.2	0.3	193
194	1.1	3.0	2.4	0.8	0.5	0.5	...	0.6	0.2	194
195	0.3	1.0	1.6	1.0	0.9	1.5	0.2	1.6	0.4	195
196	0.3	1.2	1.5	0.6	0.5	0.5	0.2	0.6	0.2	196
197	1.6	1.8	2.7	1.7	0.7	0.9	0.3	3.0	1.8	197
198	0.3	0.5	0.8	0.4	...	0.7	...	0.9	0.5	198
199	19	9.6	1.8	0.8	0.3	1.0	...	2.0	0.8	199
200	3.4	2.5	0.9	0.6	0.3	0.8	...	1.7	1.0	200
201	0.4	0.4	0.6	0.4	0.2	0.4	...	1.0	0.6	201
202	0.2	0.3	0.4	0.7	0.3	1.0	...	2.0	1.3	202
203	...	0.4	0.5	0.2	0.2	0.2	...	0.4	...	203
204	...	0.3	0.3	0.7	0.9	0.2	...	0.5	0.3	204
205	...	0.3	1.6	0.4	0.3	0.2	...	1.3	0.2	205
206	...	0.5	0.7	0.3	0.2	0.2	...	0.2	...	206
207	0.6	2.0	2.0	1.2	1.1	0.9	0.2	2.8	1.2	207
208	0.5	1.8	5.4	0.7	0.6	0.5	0.3	1.0	0.3	208
209	0.3	0.9	2.0	0.9	0.8	1.1	0.2	1.3	0.5	209
210	0.3	1.0	0.8	0.5	0.5	0.4	0.5	0.4	0.2	210
211	0.4	0.8	1.4	1.0	0.7	0.8	0.3	2.7	1.7	211
212	0.2	0.4	0.8	0.7	0.2	0.2	...	0.6	0.3	212
213	6.6	5.5	1.9	0.8	0.4	0.4	0.2	1.1	0.2	213
214	1.2	1.7	1.3	0.2	0.2	0.2	...	0.3	...	214
215	0.2	0.3	0.9	...	...	...	...	...	...	215
216	...	...	0.3	...	...	...	...	...	...	216
217	...	0.3	1.4	0.2	0.2	0.2	...	0.3	...	217
218	...	0.3	0.3	...	...	...	...	...	...	218
219	...	0.3	0.4	0.3	0.3	0.2	...	1.0	0.2	219
220	1.4	4.6	0.3	0.3	0.2	...	...	0.4	...	220
221	0.4	3.5	1.5	1.1	1.1	0.9	...	0.8	0.2	221
222	5.2	15	1.7	1.3	0.6	0.5	0.2	0.4	...	222
223	1.8	4.2	0.9	1.0	0.6	0.8	0.2	1.4	0.3	223
224	0.3	0.7	2.2	0.7	0.6	0.4	0.2	0.4	...	224
225	0.4	0.9	1.8	0.9	0.6	0.6	...	2.2	1.5	225
226	...	0.4	0.6	0.4	0.2	0.2	...	0.5	0.4	226
227	0.9	2.2	11	0.6	0.6	0.6	0.2	1.0	0.2	227
228	0.6	2.5	2.8	0.3	0.3	0.5	...	0.3	...	228
229	...	0.5	0.5	...	0.2	...	...	...	...	229
230	...	...	...	...	...	...	...	...	...	230
231	...	...	...	0.2	...	...	...	0.2	...	231
232	...	...	...	...	...	...	...	...	...	232
233	...	0.3	1.0	0.3	0.2	0.2	...	0.9	...	233
234	...	0.6	0.5	0.2	...	...	...	0.3	...	234
235	0.7	2.6	1.3	1.0	1.0	0.8	...	0.6	...	235
236	0.7	5.6	1.5	0.8	0.6	0.5	...	0.4	0.2	236
237	0.2	1.5	0.6	0.8	0.6	0.7	0.2	1.2	0.2	237
238	...	0.3	1.0	1.2	0.5	0.4	0.6	0.4	0.2	238
239	0.9	1.7	0.7	1.0	0.7	0.6	0.2	1.8	1.2	239

m/e	A	B	C	D	E	F	G	H	I	m/e
240	0.2	0.3	0.4	0.4	0.2	0.2	...	0.5	0.3	240
241	0.4	1.1	7.0	0.7	1.0	2.3	0.5	0.9	...	241
242	0.4	0.5	4.2	0.3	0.5	0.6	0.2	0.2	...	242
243	0.2	...	0.9	...	0.2	...	...	...	...	243
244	...	...	...	...	...	...	...	...	...	244
245	...	0.2	0.3	...	...	...	...	...	...	245
246	0.6	2.3	...	...	...	...	...	...	...	246
247	0.3	1.1	...	0.2	0.2	0.2	...	0.8	...	247
248	...	0.4	2.7	0.2	0.2	...	...	0.3	...	248
249	0.3	0.8	2.6	0.9	1.2	1.0	0.2	0.5	...	249
250	0.2	0.8	7.2	0.6	0.9	0.7	0.2	0.6	...	250
251	...	0.6	3.5	0.6	0.7	0.8	0.4	1.3	...	251
252	...	0.5	0.9	0.5	0.9	0.7	0.5	0.5	...	252
253	1.3	2.5	0.9	0.9	0.7	0.5	0.3	1.5	0.6	253
254	0.5	1.1	0.4	0.4	0.3	0.2	...	0.4	0.2	254
255	0.3	1.5	1.0	1.6	0.4	0.9	0.4	0.8	...	255
256	0.2	0.6	9.9	0.7	0.7	0.7	0.3	0.3	...	256
257	...	...	4.5	0.2	0.4	0.3	...	...	...	257
258	...	...	1.0	...	...	...	...	...	...	258
259	...	...	0.4	0.2	...	...	...	0.3	...	259
260	...	0.2	0.4	...	...	...	...	0.3	...	260
261	...	0.3	0.6	0.3	0.2	0.2	...	0.7	...	261
262	...	0.3	0.3	0.2	0.3	0.2	...	0.4	...	262
263	0.3	1.2	2.0	1.2	1.3	1.1	...	0.6	...	263
264	6.5	30	1.7	1.2	1.7	1.4	0.2	0.7	...	264
265	9.8	24	0.7	0.7	1.8	2.2	...	1.4	0.2	265
266	2.2	4.6	...	1.0	0.8	0.7	2.2	0.8	...	266
267	0.6	1.9	0.9	1.8	0.6	0.5	0.5	1.0	0.8	267
268	0.4	0.5	0.4	0.5	0.3	0.2	...	0.4	0.2	268
269	...	0.3	0.7	1.3	0.5	0.6	0.2	2.7	...	269
270	...	...	0.6	0.8	1.4	0.8	0.7	0.8	...	270
271	...	...	0.3	0.2	0.6	0.4	0.2	0.3	...	271
272	...	...	...	...	...	...	...	0.3	...	272
273	...	...	...	0.2	0.2	...	...	0.2	...	273
274	...	...	...	0.2	...	...	...	0.2	...	274
275	...	...	0.3	0.4	0.2	...	...	0.4	...	275
276	...	...	0.8	0.7	...	...	...	0.2	...	276
277	0.2	...	2.1	2.0	1.1	1.0	0.3	0.8	...	277
278	0.3	2.9	9.0	3.3	0.5	0.7	1.0	0.6	0.2	278
279	...	0.4	3.4	9.6	0.5	0.6	0.6	1.2	0.4	279
280	...	0.8	0.6	2.7	0.6	0.4	...	0.5	0.3	280
281	...	0.4	2.0	1.1	0.6	0.5	...	0.6	0.9	281
282	...	0.2	0.6	0.9	0.3	0.2	...	0.3	0.3	282
283	...	0.3	0.8	0.9	0.9	1.3	...	0.7	0.2	283
284	...	0.3	0.4	1.7	0.5	0.6	...	0.2	...	284
285	...	...	...	0.4	0.2	...	...	...	...	285
286	...	...	...	...	...	...	...	...	...	286
287	...	...	...	...	0.2	0.2	...	...	...	287
288	...	...	1.5	...	...	...	...	...	...	288
289	...	...	0.7	0.3	0.2	0.2	...	6.1	...	289
290	...	...	...	0.2	0.2	...	...	1.8	...	290
291	...	0.8	1.4	1.1	1.6	1.3	0.2	0.6	0.2	291
292	...	0.8	0.5	1.7	0.9	0.8	0.2	0.5	...	292
293	...	0.9	0.3	1.5	0.6	0.5	...	0.9	...	293
294	...	0.5	...	0.9	0.5	0.4	...	0.4	...	294
295	0.4	0.8	1.2	1.6	0.6	0.5	...	0.3	0.3	295
296	22	7.7	0.5	0.9	0.8	0.3	...	...	...	296
297	6.7	3.7	0.6	1.0	0.7	1.1	...	0.5	...	297
298	1.1	1.6	...	1.1	0.9	0.9	...	0.2	...	298
299	0.2	0.3	...	0.3	0.3	0.2	...	...	...	299
300			...	...	...	...	...	0.3	...	300
301			0.8	0.2	0.2	0.2	...	0.2	...	301
302			...	0.2	...	0.2	...	0.3	...	302
303			...	0.4	0.3	0.3	...	0.6	...	303
304			0.3	0.5	0.2	0.2	...	0.6	...	304
305			0.8	3.4	2.5	2.5	0.3	0.9	...	305
306			68	2.6	1.6	1.7	0.8	1.3	...	306
307			39	2.1	2.4	2.2	0.3	13.2	0.2	307
308			7.4	0.7	1.2	1.0	...	5.2	...	308
309			1.3	2.2	0.8	0.7	...	23.9	0.2	309
310			0.3	0.8	0.5	0.4	...	5.7	...	310
311			...	2.8	0.6	0.4	0.3	1.4	0.5	311
312			...	5.4	1.7	1.7	0.2	...	0.2	312
313			...	1.3	0.5	0.4	...	...	0.2	313
314			...	0.2	...	...	...	...	...	314
315			...	0.3	...	...	...	...	...	315
316			1.4	0.3	...	...	0.2	...	...	316
317			0.4	0.2	0.2	...	...	6.4	0.3	317
318			...	0.2	0.4	0.3	...	1.9	0.2	318
319			...	1.3	1.5	1.7	0.2	0.4	0.4	319

m/e	C	D	E	F	G	H	I	m/e
320	0.6	0.8	4.4	5.0	...	0.2	0.2	320
321	0.4	0.7	9.4	9.5	...	0.2	0.3	321
322	...	0.6	2.6	2.4	...	...	0.2	322
323	1.8	1.9	0.8	0.8	...	0.3	0.2	323
324	0.5	0.7	0.5	0.3	0.2	0.3	...	324
325	...	1.1	1.4	1.7	...	0.4	...	325
326	...	0.6	1.3	1.5	0.3	...	...	326
327	...	0.2	0.5	0.3	...	...	...	327
328	...	...	...	...	...	...	...	328
329	...	...	...	...	...	...	...	329
330	...	0.2	...	...	...	...	...	330
331	...	0.2	...	...	...	...	...	331
332	...	0.4	...	...	...	0.2	...	332
333	...	1.3	5.3	6.6	1.4	0.2	...	333
334	...	7.5	2.6	3.6	2.4	0.4	1.5	334
335	...	23	0.8	0.9	0.5	15	0.9	335
336	0.8	6.1	0.4	0.3	...	4.0	0.3	336
337	0.6	1.8	0.9	1.0	...	1.1	3.8	337
338	64	0.9	0.5	0.4	...	1.2	1.1	338
339	17.4	0.4	3.1	3.4	...	57	1.8	339
340	2.6	0.3	1.0	0.9	...	14	0.5	340
341	0.3	...	0.3	0.2	...	2.0	0.2	341
342		...	...	...	...	0.3	...	342
343		...	0.6	0.9	...	...	...	343
344		0.3	0.3	0.3	...	...	...	344
345		0.3	...	...	...	0.3	0.2	345
346		0.4	...	...	...	...	...	346
347		0.3	1.5	1.5	...	2.2	...	347
348		3.5	1.6	2.0	...	0.8	0.3	348
349		1.4	1.2	...	...	0.4	0.2	349
350		0.6	0.6	0.4	...	...	0.3	350
351		1.4	1.7	2.0	...	...	0.2	351
352		0.8	18	16	...	...	...	352
353		0.7	9.5	7.2	...	0.3	1.4	353
354		0.2	2.1	1.4	...	...	0.5	354
355		...	0.3	0.2	...	0.3	...	355
356		...	...	...	...	...	...	356
357		...	...	...	...	...	...	357
358		...	0.2	0.2	...	...	...	358
359		...	0.2	0.2	...	...	0.2	359
360		...	0.3	0.2	...	...	...	360
361		...	10	9.5	...	...	...	361
362		0.3	4.3	4.1	...	...	...	362
363		0.2	2.7	3.3	...	0.5	...	363
364		0.2	0.8	0.9	...	0.3	...	364
365		1.9	0.5	0.6	...	5.3	0.4	365
366		0.9	0.6	1.0	5.5	2.5	0.2	366
367		0.3	0.3	0.3	1.5	53	0.5	367
368		0.2	0.4	...	0.3	14	0.3	368
369		...	...	...	...	2.4	...	369
370		0.2	...	...		0.3	...	370
371		...	...	...		...	...	371
372		...	...	...		...	...	372
373		...	...	...		...	0.2	373
374		...	0.7	0.6		...	0.3	374
375		...	6.1	5.6		...	1.4	375
376		...	11	14		...	0.5	376
377		...	3.1	4.1		...	0.5	377
378		1.5	1.2	2.2		...	0.2	378
379		0.2	0.3	0.5		...	...	379
380		94	...	...		...	...	380
381		26	0.4	...		0.3	3.1	381
382		4.0	...	...		0.3	1.0	382
383		0.5	...	...		...	0.2	383
384		...	...	...		...	...	384
385		...	...	...		...	...	385
386		...	...	...		...	...	386
387		...	...	...		...	...	387
388		...	...	...		...	...	388
389		...	...	...		...	...	389
390		...	...	...		...	...	390
391		...	0.5	0.4		...	...	391
392		...	12	25		...	0.4	392
393		...	23	20		...	0.3	393
394		0.6	5.9	5.7		...	0.9	394
395		0.2	1.0	0.9		0.3	0.3	395
396		3.0	...	...		0.3	...	396
397		0.8	...	...		0.5	...	397
398		0.2	...	...		0.2	...	398
399		...	...	...		...	...	399

m/e	E	F	I	m/e
400	...	...	...	400
401	...	...	...	401
402	...	...	...	402
403	...	...	...	403
404	...	...	...	404
405	...	...	...	405
406	...	0.2	...	406
407	0.3	1.5	...	407
408	99.2	75	...	408
409	31.7	24	0.5	409
410	4.0	4.5	0.2	410
411	0.6	0.8	...	411
412	...		...	412
413			...	413
414			...	414
415			...	415
416			...	416
417			...	417
418			...	418
419			...	419
420			...	420
421			...	421
422			...	422
423			0.3	423
424			10	424
425			3.5	425
426			0.7	426